STATE OF MONTANA
NATURAL RESOURCE DAMAGE PROGRAM

AQUATIC RESOURCES
INJURY ASSESSMENT REPORT
APPENDICES A - G

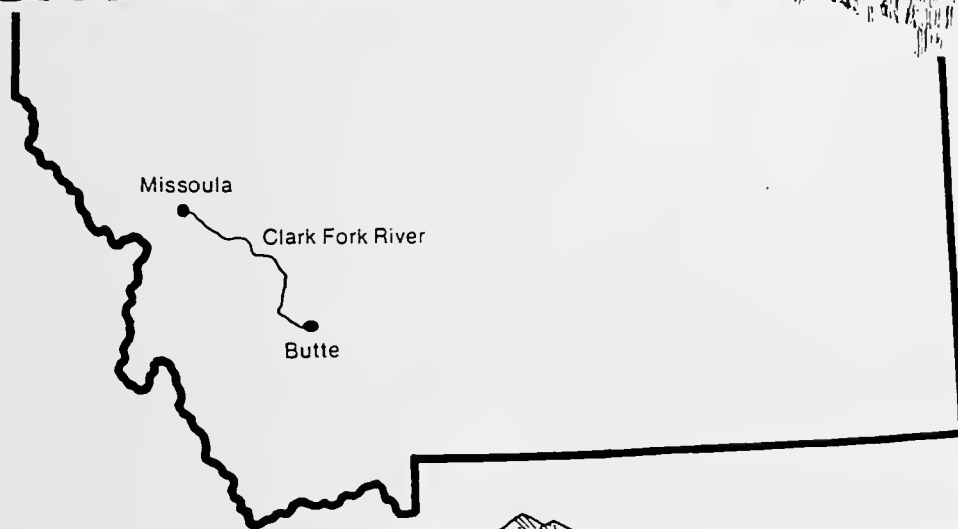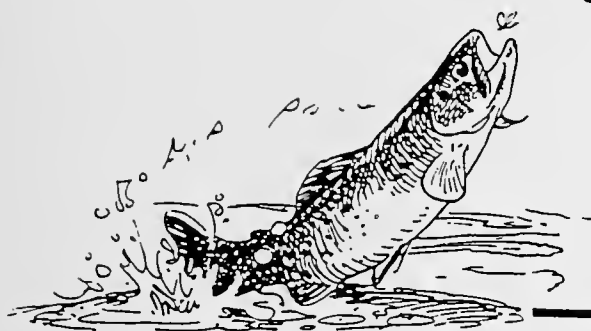## UPPER CLARK FORK RIVER BASIN

JUNE 1993

Missoula

Clark Fork River

Butte

AQUATIC RESOURCES
INJURY ASSESSMENT REPORT

UPPER CLARK FORK RIVER BASIN

APPENDICES A - G

Prepared by:

Joshua Lipton, Ph.D., Project Manager
RCG/Hagler, Bailly, Inc.

Contributors:

Harold Bergman, Ph.D., University of Wyoming
Don Chapman, Ph.D., Don Chapman Consultants, Inc.
Tracy Hillman, Ph.D., Don Chapman Consultants, Inc.
Mark Kerr, Montana Natural Resource Damage Program
Johnnie Moore, Ph.D., University of Montana
Dan Woodward, U.S. Fish and Wildlife Service

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AQUATIC RESOURCES INJURY REPORT

APPENDIX A

*Surface Water Sampling Conducted by Montana
Natural Resource Damage Program (NRDP)*

Prepared by:

Mark Kerr, NRDP

1.0   INTRODUCTION

This appendix summarizes the collection and analysis of surface water data by the State of Montana Natural Resource Damage Program (NRDP).

2.0   OBJECTIVES

As stated in Assessment Plan: Part II, Clark Fork Basin NPL Sites, Montana, specific objectives of this sampling were:

- ▶ To characterize concentrations of hazardous substances at fish population sites in the upper Clark Fork Basin and at control streams.

- ▶ To characterize concentrations of hazardous substances in Boulder Batholith-type streams in the Butte area (Blacktail Creek, Yankee Doodle Creek, and upper Silver Bow Creek) to provide information on baseline water quality conditions.

A third objective was to collect data to support the use of existing data for the surface water injury determination. A review of existing data identified two subtasks which would improve the usability of existing data. These subtasks were:

- ▶ To evaluate the comparability of data collected by different sampling methods (i.e., grab-sampling and composite sampling).

- ▶ To evaluate the comparability of different methods (dissolved, Montana total recoverable, and EPA total recoverable concentrations) of metals analysis.

3.0   METHODS

3.1   Sample Site Selection

Surface water sampling sites were defined primarily by the location of fisheries population "IFIM" sites (see Appendix G). One site on Silver Bow Creek, five sites on the Clark Fork River, and their matching sites on control streams (Bison Creek, Big Hole River, Ruby River, and Rock Creek) were sampled. Three additional sites were sampled at control streams to assess changes in metals concentrations over distance. Many of the streams selected as controls for Silver Bow Creek and the Clark Fork River had little or no existing metals concentrations data.

Silver Bow Creek headwaters streams (upper Silver Bow Creek, Yankee Doodle Creek, and Blacktail Creek) were sampled to characterize metals concentrations in streams draining the Boulder Batholith. The first two streams were tributaries to Silver Bow Creek before construction of the Yankee Doodle Tailings ponds and the Berkeley Pit eliminated parts of their channels. Blacktail Creek is presently the major tributary to the headwaters of Silver Bow Creek.

Samples were collected from the following locations (Figure A-1):

1. Silver Bow Creek near Ramsay (SBC 1)
2. Clark Fork River at Deer Lodge (CFR 1)
3. Clark Fork River at Gold Creek (CFR 2)
4. Clark Fork River at Bearmouth (CFR 3)
5. Clark Fork River at Beavertail Hill (CFR 4)
6. Clark Fork River at Turah (CFR 5)
7. Bison Creek (BC 1) at Elk Park - match site for SBC 1
8   Ruby River (RR 1) below Ruby Reservoir - match site for CFR 1 and downstream Ruby River IFIM site
9. Ruby River (RR 2) above Ruby Reservoir - upstream Ruby River IFIM site
10. Big Hole River (BH 1) at Kalsta Ranch - match site for CFR 2 and upstream Big Hole River IFIM site
11. Big Hole River (BH 2) at Notch Bottom - downstream Big Hole River IFIM site
12. Rock Creek (RC 1) near Stonehenge - match site for CFR 3 and upstream Rock Creek IFIM site
13. Rock Creek (RC 2) above mouth - match site for CFR 4 and CFR 5, and downstream Rock Creek IFIM site
14. Flint Creek below Douglas Creek (FC 1)
15. Silver Bow Creek above Yankee Doodle Tailings - Boulder Batholith stream and control for Silver Bow Creek (SBC 2)
16. Yankee Doodle Creek - Boulder Batholith stream and control for Silver Bow Creek (YD 1)
17. Blacktail Creek - Boulder Batholith stream and control for Silver Bow Creek (BT 1)

Most sites were sampled six times during the spring runoff months of April and May, 1992. The three Silver Bow Creek headwater streams, and the Silver Bow Creek and Bison Creek IFIM sites, were only sampled three times in April and May. Sites were sampled every seven to nine days. At most IFIM sites, a sampling transect was established within the approximately 100 meter long fish population and habitat assessment reach. On the Clark Fork River at Turah, the Ruby River above Ruby Reservoir, and Rock Creek near Stonehenge, sampling transects were not located on the
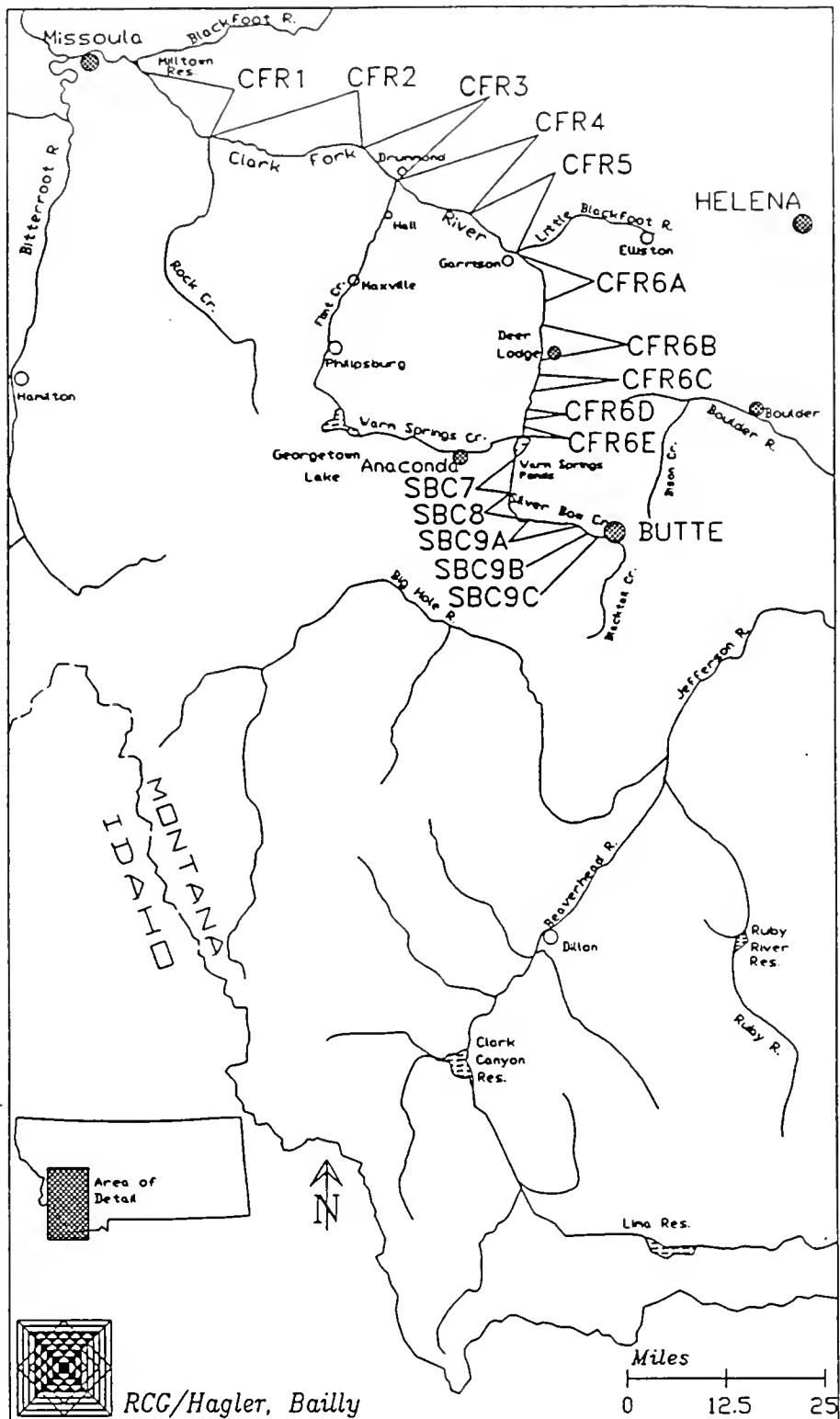
Figure A-1.   Surface Water Sampling Site Locations.

IFIM sites due to difficult access. Except for several occasions where circumstances required moving the transect, samples were collected at the same transect each time.

3.2   Field Sampling Protocols

Samples were collected as channel-integrated and depth-integrated composite samples. This method was used to approximate average water quality conditions across the stream channel (irrespective of flow velocity). For the purposes of evaluating aquatic life criteria, a concentration representing average conditions was deemed more appropriate that a flow-weighted concentration.

At each sample location, a sampling and flow-gauging transect was established. After gauging streamflow, surface water samples were collected across the transect. At each point in the transect, a sample of 250 mls + 50 mls was collected. These samples were composited into a bucket. Composited samples were subsampled for EPA TR metals and total suspended solids/specific conductance with representative splits being provided to ARCO representatives. After subsampling, samples were filtered from the compositing bucket for dissolved metals.

3.3   Quality Assurance/Quality Control

Quality control (QC) samples were collected to evaluate potential sample contamination, and to evaluate consistency and replicability of sample collection methods. QC samples for measuring sample contamination included trip blanks, filter blanks, and field decontamination (rinseate) blanks. Grab samples and field duplicate samples were collected to validate sample collection methods. Split samples were collected to validate subsampling (i.e., sample splitting) techniques.

To ensure that samples were analyzed "blind" by the laboratory, samples were assigned a six digit field number. The first three numbers were a generated random number; the second three numbers represented the month and date of sample collection. Samples were thus checked into the laboratory as a six digit number (i.e., 929-505) with no site identification information provided. Sample numbers were entered onto a sample tracking form in the field, along with corresponding site identification information and other pertinent information. Sample numbers were also entered on chain-of-custody forms.

Samples were checked into the lab the morning after the second day of field work. Requested analyses were entered on chain-of-custody forms, and chain-of-custody forms were segregated by analysis type (i.e., total recoverable metals, dissolved metals, TSS/SC, etc). Samples were collected for the following analyses: Cd, Cu, Pb and Zn (EPA total

recoverable, Montana total recoverable, dissolved); Ca and Mg (dissolved); total suspended solids (TSS) and specific conductance (SC). The laboratory sample number assigned to each field sample was entered on the COC form after samples were logged into the laboratory.

3.4   Laboratory Analyses

All samples were analyzed for EPA TR and dissolved metals. Samples for EPA TR metals were digested following EPA method 200.2. Both ICP and GFAA analytical methods were used in the analysis of total recoverable and dissolved metals. ICP analyses followed EPA Method 200.7. GFAA analysis followed EPA Methods 213.2 (Cd), 220.2 (Cu), 239.2 (Pb), and 289.2 (Zn). All analyses for cadmium and lead were made using GFAA only. Copper and zinc were analyzed by both ICP and GFAA. The ICP analysis was generally made first to identify samples with high copper and zinc concentrations. Samples below the ICP detection limits were then reanalyzed by GFAA. Because of overlapping analytical ranges, many samples have both an ICP and a GFAA measured concentration. During the analysis of zinc, the initial ICP detection limit of 5 ppb was redetermined as 2 ppb. Therefore, many samples which were initially analyzed by ICP at the 5 ppb detection limit were reanalyzed at the 2 ppb detection limit (3 ppb for EPA total recoverable zinc). Samples with zinc concentrations less than 2 ppb were subsequently reanalyzed by GFAA.

The Montana total recoverable (MT TR) analysis is made on a field preserved sample which undergoes no additional laboratory digestion. The settled sample is aspirated directly from the sample bottle for ICP or GFAA analysis.

Calcium and magnesium were analyzed on filtered samples using ICP.

Detection limits for the various metals and methods are as follows:

- ► Cadmium - 0.2 ppb (GFAA)
- ► Copper - 10 ppb (ICP); 1 ppb (GFAA) (100 ppb high standard)
- ► Lead - 1 ppb (GFAA)
- ► Zinc - 2 ppb (ICP); 1 ppb (GFAA)
- ► Calcium - 1 ppm (ICP)
- ► Magnesium - 1 ppm (ICP).

3.5   Statistical Methods

To determine comparability of different sample collection techniques and different sample digestion techniques, the relative percent difference (RPD) was calculated for

paired samples (i.e., composite/grab, EPA TR/MT TR). An RPD of 20% was used as the threshold for comparability.

4.0   RESULTS

4.1   Metals Concentrations

Results of EPA TR, MT TR, and dissolved metals analyses are summarized in Tables A-1 through A-3. Table A-1 summarizes concentrations of cadmium, copper, lead and zinc at sites in Silver Bow Creek and the Clark Fork River. Table A-2 summarizes concentrations at IFIM sites in Clark Fork River reference streams (Big Hole River, Rock Creek, and Ruby River). Table A-3 summarizes concentrations in the Silver Bow Creek headwaters streams (upper Silver Bow Creek, Yankee Doodle Creek, and Blacktail Creek); Bison Creek (Silver Bow Creek IFIM reference stream), and in Flint Creek (an IFIM stream which was not matched to a Clark Fork River site). For presentation purposes, copper, lead, and zinc data are rounded to the nearest 1 ppb; cadmium is rounded to the nearest 0.1 ppb.

4.2   Field Data and Other Laboratory Analyses

Field data which were collected included streamflow, water temperature, and pH. Samples were collected for laboratory analysis of total suspended solids (TSS) and specific conductance (SC). Field, TSS and SC data are summarized in Tables A-4 through A-6.

4.3   Comparison of EPA and MT Total Recoverable Concentrations

One objective of this sampling project was to compare Montana total recoverable (MT TR) metals concentrations to EPA total recoverable (EPA TR) metals concentrations. This objective is important because:

► EPA recommends that ambient water quality criteria be evaluated on the basis of an EPA TR digestion method.

► Much of the existing dataset for Silver Bow Creek and the Clark Fork River has been collected by the State of Montana DHES and analyzed using the MT TR method.

Table A-1

EPA Total Recoverable (EPA TR), Montana Total Recoverable (MT TR) and Dissolved Hazardous Substance Concentrations in Silver Bow Creek and the Clark Fork River[1]

Sampling Location	Date	Cadmium			Copper			Lead			Zinc		
		EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
Silver Bow Creek near Ramsay	04/06/92	2.1	1.9	1.0	244	233	75	28	27	ND	559	585	304
	04/24/92	1.8	1.4	0.7	130	141	53	10	10	1	449	494	219
	05/12/92	1.7	1.6	1.2	94	111	52	5	5	ND	581	648	475
Clark Fork River at Deer Lodge	04/05/92	0.3	ND	ND	37	35	10	3	3	ND	52	41	11
	04/15/92	0.2	NM	ND	26	NM	7	2	NM	ND	30	NM	11
	04/23/92	ND	ND	ND	15	14	7	ND	1	ND	17	16	6
	05/03/92	ND	NM	ND	26	NM	9	2	NM	ND	39	NM	11
	05/11/92	ND	ND	ND	15	14	8	1	1	ND	14	14	4
	05/20/92	0.3	NM	ND	11	NM	6	ND	NM	ND	13	NM	13
Clark Fork River at Gold Creek	04/05/92	0.4	0.3	ND	53	48	5	7	6	ND	74	54	7
	04/15/92	0.2	NM	ND	22	NM	4	3	NM	ND	20	NM	3
	04/23/92	ND	ND	ND	19	15	5	2	1	1	18	15	4
	05/03/92	ND	NM	ND	16	NM	4	3	NM	ND	26	NM	2
	05/11/92	ND	ND	ND	13	12	6	2	1	ND	15	13	2
	05/20/92	ND	NM	ND	7	NM	3	ND	NM	ND	10	NM	3
Clark Fork River at Bearmouth	04/05/92	0.2	ND	ND	33	29	3	7	6	ND	65	41	6
	04/15/92	0.2	NM	ND	17	NM	4	3	NM	ND	24	NM	3
	04/23/92	ND	ND	ND	22	22	3	4	4	1	30	26	5
	05/03/92	ND	NM	ND	21	NM	4	3	NM	ND	29	NM	3
	05/11/92	ND	ND	ND	8	7	5	ND	ND	ND	11	10	3
	05/20/92	ND	NM	ND	4	NM	3	ND	NM	ND	5	NM	4
Clark Fork River at Beavertail Hill	04/05/92	0.2	0.2	ND	33	28	3	6	6	ND	69	42	4
	04/15/92	ND	NM	ND	18	NM	4	3	NM	ND	23	NM	3
	04/23/92	ND	ND	ND	18	17	3	3	3	ND	28	25	2
	05/03/92	ND	NM	ND	17	NM	4	3	NM	ND	30	NM	2
	05/11/92	ND	ND	ND	8	7	5	1	ND	ND	8	8	2
	05/20/92	ND	NM	ND	5	NM	3	ND	NM	ND	4	NM	2
Clark Fork River at Turah	04/05/92	ND	ND	ND	45	15	2	7	3	ND	85	24	6
	04/15/92	ND	NM	ND	11	NM	2	2	NM	ND	16	NM	2
	04/23/92	ND	ND	ND	12	11	2	2	2	ND	17	12	2
	05/03/92	ND	NM	ND	9	NM	2	4	NM	ND	20	NM	2
	05/11/92	ND	ND	ND	4	3	2	ND	NM	ND	8	8	2
	05/20/92	0.2	NM	ND	3	NM	ND	ND	NM	ND	5	NM	ND

[1] Concentrations in μg/l. ND = concentrations below the detection limit (cadmium = 0.2 μg/l; copper, lead and zinc = 1 ppb). NM = not measured.

Table A-2

EPA Total Recoverable (EPA TR), Montana Total Recoverable (MT TR) and Dissolved Hazardous Substance Concentrations in the Big Hole River, Rock Creek, and the Ruby River[1].

Sampling Location	Date	Cadmium			Copper			Lead			Zinc		
		EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
Big Hole River at Kalsta Ranch	04/06/92	ND	ND	ND	2	ND	ND	1	ND	ND	13	3	2
	04/14/92	ND	NM	ND	4	NM	ND	1	NM	ND	3	NM	3
	04/24/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	2	1	ND
	05/02/92	ND	NM	ND	1	NM	ND	2	NM	ND	7	NM	1
	05/12/92	ND	ND	ND	2	1	2	ND	ND	ND	5	2	ND
	05/21/92	ND	NM	ND	3	NM	ND	ND	NM	ND	4	NM	ND
Big Hole River at Notch Bottom	04/06/92	ND	ND	ND	1	2	ND	1	ND	ND	13	3	27
	04/14/92	ND	NM	ND	3	NM	ND	2	NM	ND	4	NM	4
	04/24/92	ND	ND	ND	1	ND	ND	ND	ND	ND	3	1	ND
	05/02/92	ND	NM	ND	ND	NM	ND	2	NM	ND	4	NM	1
	05/12/92	ND	ND	ND	3	2	2	ND	ND	ND	3	1	ND
	05/21/92	ND	NM	ND	ND	NM	ND	ND	NM	ND	3	NM	ND
Rock Creek near Stonehenge	04/06/92	ND	ND	ND	ND	ND	ND	2	ND	ND	8	ND	5
	04/14/92	ND	NM	ND	ND	NM	ND	1	NM	ND	8	NM	3
	04/24/92	ND	ND	ND	2	2	ND	ND	ND	ND	2	ND	2
	05/02/92	ND	NM	ND	2	NM	ND	1	NM	ND	4	NM	ND
	05/12/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	7
	05/21/92	ND	NM	ND	ND	NM	ND	ND	NM	ND	2	NM	ND
Rock Creek near mouth	04/06/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	4	1	4
	04/14/92	ND	NM	ND	ND	NM	ND	ND	NM	ND	2	NM	4
	04/24/92	ND	ND	ND	3	2	2	1	ND	ND	5	1	ND
	05/02/92	ND	NM	ND	ND	NM	ND	1	NM	ND	4	NM	ND
	05/12/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	ND
	05/21/92	ND	NM	ND	ND	NM	ND	ND	NM	ND	2	NM	ND
Ruby River above Ruby Reservoir	04/06/92	ND	ND	ND	1	ND	ND	3	ND	ND	13	7	5
	04/14/92	0.2	NM	ND	8	NM	3	3	NM	ND	6	NM	2
	04/24/92	ND	ND	ND	2	1	ND	2	2	ND	8	7	ND
	05/02/92	ND	NM	ND	3	NM	ND	4	NM	ND	15	NM	2
	05/12/92	ND	ND	ND	2	ND	ND	2	1	ND	10	5	ND
	05/21/92	ND	NM	ND	3	NM	ND	3	NM	ND	13	NM	ND
Ruby River below Ruby Reservoir	04/06/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	12	ND	4
	04/14/92	ND	NM	ND	ND	ND	ND	1	NM	ND	2	NM	4
	04/24/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	1	ND
	05/02/92	ND	NM	ND	ND	ND	ND	ND	NM	ND	3	NM	2
	05/12/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	ND
	05/21/92	ND	NM	ND	ND	ND	ND	ND	NM	ND	2	NM	ND

[1] Concentrations in $\mu g/l$. ND = concentrations below the detection limit (cadmium = 0.2 $\mu g/l$; copper, lead and zinc = 1 ppb). NM = not measured.

RCG/Hagler, Bailly, Inc.

Table A-3
EPA Total Recoverable (EPA TR), Montana Total Recoverable (MT TR) and Dissolved
Hazardous Substance Concentrations In Silver Bow Creek Headwater Streams (Upper Silver Bow Creek,
Yankee Doodle Creek, and Blacktail Creek), Bison Creek, and Flint Creek[1]

Sampling Location	Date	Cadmium			Copper			Lead			Zinc		
		EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
Silver Bow Creek above Yankee Doodle Tailings	04/15/92	ND	NM	ND	2	NM	ND	ND	NM	ND	3	NM	3
	05/02/92	ND	NM	ND	2	NM	ND	21	NM	ND	8	NM	ND
	05/21/92	ND	NM	ND	1	NM	1	ND	NM	ND	2	NM	ND
Yankee Doodle Creek above Yankee Doodle Tailings	04/15/92	ND	NM	ND	3	NM	ND	ND	NM	ND	2	NM	2
	05/02/92	ND	NM	ND	2	NM	ND	3	NM	ND	11	NM	ND
	05/21/92	ND	NM	ND	5	NM	ND	ND	NM	ND	8	NM	1
Blacktail Creek at Thompson Park	04/15/92	ND	NM	ND	4	NM	2	ND	NM	ND	6	NM	3
	05/02/92	ND	NM	ND	5	NM	2	1	NM	ND	9	NM	ND
	05/21/92	0.3	NM	ND	8	NM	3	ND	NM	ND	6	NM	1
Bison Creek at Elk Park	04/15/92	ND	ND	ND	7	5	5	1	ND	ND	20	20	16
	05/02/92	ND	ND	ND	17	4	2	1	ND	ND	12	6	6
	05/21/92	ND	ND	ND	4	3	8	ND	ND	ND	8	9	3
Flint Creek	04/05/92	ND	ND	ND	6	4	ND	15	14	ND	43	31	7
	04/15/92	ND	NM	ND	3	NM	ND	7	NM	ND	19	NM	4
	04/23/92	ND	ND	ND	3	3	ND	8	9	ND	19	14	ND
	05/03/92	ND	NM	ND	2	NM	ND	4	NM	ND	13	NM	ND
	05/11/92	ND	ND	ND	3	3	1	4	4	ND	11	9	ND
	05/20/92	ND	NM	ND	4	NM	ND	11	NM	ND	25	NM	2

[1] Concentrations in $\mu g/l$. ND = concentrations below the detection limit (cadmium = 0.2 $\mu g/l$; copper, lead and zinc = 1 ppb). NM = not measured.

Table A-4
Flow, Field Temperature (Temp), Field pH (pH), Total Suspended Solids (TSS), Specific Conductance (SC), Calcium (Ca), Magnesium (Mg) and Hardness in Silver Bow Creek and the Clark Fork River[1]

Location	Date	Flow (cfs)	Temp (°C)	pH	TSS (mg/l)	SC (umhos at 25° C)	Ca (mg/l)	Mg (mg/l)	Hardness (mg/l CaCO$_3$)
Silver Bow	04/06/92	34	7	8.16	24.2	402	42.4	9.4	145
Creek near	04/24/92	20	12	8.06	11.5	496	46.4	9.9	157
Ramsay	05/12/92	15	7	7.09	6.6	488	46.6	10.2	158
Clark Fork	04/05/92	210	7	7.32	16.2	551	69.9	15.9	240
River at	04/15/92	164	9	7.39	8.0	574	73.3	17.0	253
Deer Lodge	04/23/92	172	8	7.95	4.2	564	76.0	17.0	260
	05/03/92	184	10	7.73	NM	497	66.2	14.5	225
	05/11/92	126	13	8.62	4.4	476	62.8	13.5	212
	05/20/92	31	16	8.19	NM	537	66.7	13.7	223
Clark Fork	04/05/92	380	6	7.74	36.9	473	59.6	13.2	203
River at	04/15/92	364	11	8.07	NM	455	58.1	12.9	198
Gold Creek	04/23/92	431	4	7.68	86.7	427	55.6	12.2	189
	05/03/92	419	15	8.30	NM	400	51.7	11.2	175
	05/11/92	255	13	8.24	7.4	405	53.1	11.5	180
	05/20/92	142	13	7.80	NM	412	52.8	11.5	179
Clark Fork	04/05/92	NM	7	7.94	36.8	479	61.6	14.9	215
River at	04/15/92	NM	8	8.27	NM	458	60.6	14.6	211
Bearmouth	04/23/92	NM	6	7.80	23.1	451	60.7	14.0	209
	05/03/92	NM	12	8.11	NM	434	58.3	13.5	201
	05/11/92	NM	11	7.77	2.8	498	66.0	15.6	229
	05/20/92	109	16	8.01	NM	606	82.4	20.4	290
Clark Fork	04/05/92	NM	7	7.70	33.8	475	62.4	14.9	217
River at	04/15/92	NM	13	7.24	NM	472	61.6	15.0	216
Beavertail	04/23/92	NM	7	7.70	20.0	449	60.9	14.1	210
Hill	05/03/92	NM	12	8.34	NM	430	57.8	13.7	201
	05/11/92	NM	11	7.93	2.1	495	66.2	15.9	231
	05/20/92	NM	17	8.46	NM	558	73.7	19.4	264
Clark Fork	04/05/92	852	7	7.32	24.4	402	46.7	11.3	163
River at	04/15/92	830	13	8.16	NM	331	42.3	10.7	150
Turah	04/23/92	911	7	7.28	14.1	311	40.2	9.7	140
	05/03/92	1314	10	8.28	NM	247	30.6	7.7	108
	05/11/92	1205	10	7.76	7.6	222	26.6	6.9	95
	05/20/92	845	15	8.24	NM	217	25.7	7.0	93

[1] Concentrations in μg/l. ND = concentrations below the detection limit (cadmium = 0.2 μg/l; copper, lead and zinc = 1 ppb). NM = not measured.

Table A-5
Flow, Field Temperature (Temp), Field pH (pH), Total Suspended Solids (TSS), Specific Conductance (SC), Calcium (Ca), Magnesium (Mg), and Hardness for Big Hole River, Rock Creek, and Ruby River[1,2]

Location	Date	Flow (cfs)	Temp (°C)	pH	TSS (mg/l)	SC (umhos at 25° C)	Ca (mg/l)	Mg (mg/l)	Hardness (mg/l CaCO₃)
Big Hole River at Kalsta Ranch	04/06/92	930	5	8.08	13.6	132	12.5	3.0	44
	04/14/92	874	10	8.06	NM	140	12.6	3.1	44
	04/24/92	874	3	7.09	7.0	132	12.4	3.0	43
	05/02/92	1355	10	7.70	NM	109	9.2	2.1	32
	05/12/92	1028	8	7.75	3.9	109	11.3	2.6	39
	05/21/92	1058	14	8.58	NM	114	11.9	2.9	42
Big Hole River at Notch Bottom	04/06/92	NM	6	8.18	13.2	139	14.1	3.4	49
	04/14/92	NM	9	7.71	NM	156	14.2	3.4	49
	04/24/92	NM	5	7.15	7.1	137	14.1	3.3	49
	05/02/92	NM	12	7.72	NM	110	10.9	2.4	37
	05/12/92	NM	10	7.84	5.7	123	13.3	2.9	45
	05/21/92	NM	14	8.60	NM	129	15.1	3.4	52
Rock Creek near Stonehenge	04/06/92	340	7	7.56	9.7	118	11.7	3.8	45
	04/14/92	332	11	8.42	NM	124	12.3	3.7	46
	04/24/92	NM	5	8.44	4.8	107	10.6	3.4	40
	05/02/92	NM	10	7.52	NM	85	8.2	2.6	31
	05/12/92	NM	10	8.38	6.8	82	8.5	2.7	32
	05/21/92	NM	12	8.15	NM	97	10.1	3.2	38
Rock Creek near mouth	04/06/92	378	7	7.77	8.9	128	11.9	4.1	47
	04/14/92	354	12	7.55	NM	155	12.3	3.9	47
	04/24/92	445	5	8.43	18.8	112	11.2	3.6	43
	05/02/92	689	9	8.25	NM	100	8.7	2.8	33
	05/12/92	771	9	8.48	5.1	86	8.9	2.8	34
	05/21/92	619	12	8.15	NM	98	10.3	3.3	39
Ruby River above Ruby Reservoir	04/06/92	122	6	8.26	57.6	573	71.4	22.9	273
	04/14/92	126	8	7.74	NM	573	72.2	22.9	275
	04/24/92	116	12	7.99	88.2	556	69.8	22.1	265
	05/02/92	201	12	8.43	NM	420	53.3	15.5	197
	05/12/92	206	12	8.36	55.8	452	57.9	17.0	215
	05/21/92	248	13	8.10	NM	434	54.0	15.8	200
Ruby River below Ruby Reservoir	04/06/92	19	7	8.06	5.7	675	80.4	28.4	318
	04/14/92	11	7	8.24	NM	649	76.2	27.3	303
	04/24/92	9	9	8.20	13.8	626	76.1	26.8	300
	05/02/92	13	15	8.07	NM	603	71.5	26.0	286
	05/12/92	88	11	8.38	9.2	624	74.0	25.6	290
	05/21/92	102	12	8.15	NM	611	71.6	24.0	278

[1] Concentrations in $\mu g/l$. ND = concentrations below the detection limit (cadmium = 0.2 $\mu g/l$; copper, lead and zinc = 1 ppb). NM = not measured.

Table A-6
Flow, Field Temperature (Temp), Field pH (pH), Total Suspended Solids (TSS) and Specific Conductance (SC) for Silver Bow Creek Headwater Streams (Upper Silver Bow Creek, Yankee Doodle Creek, and Blacktail Creek), Bison Creek, and Flint Creek[1,2]

Location	Date	Flow (cfs)	Temp (°C)	pH	TSS (mg/l)	SC (umhos at 25° C)	Ca (mg/l)	Mg (mg/l)	Hardness (mg/l CaCO₃)
Silver Bow Creek above Yankee Doodle Tailings	04/15/92	0.2[3]	3	8.04	1.1	251	32.0	7.1	109
	05/02/92	0.2[3]	3	7.68	1.2	246	32.7	7.3	112
	05/21/92	0.2[3]	7	7.91	1.8	270	33.6	7.6	115
Yankee Doodle Creek above Yankee Doodle Tailings	04/15/92	0.5[3]	5	6.73	4.5	144	15.8	3.3	53
	05/02/92	0.1[3]	5	7.38	5.1	160	14.8	3.2	50
	05/21/92	0.1[3]	10	7.15	3.9	140	14.6	3.2	50
Blacktail Creek at Thompson Park	04/15/92	2	6	6.94	3.1	164	18.9	4.2	64
	05/02/92	2	5	7.86	1.4	194	21.1	4.7	72
	05/21/92	1	7	8.15	5.8	199	24.2	5.0	81
Bison Creek at Elk Park	04/06/92	9	4	8.10	4.4	121	12.0	2.8	41
	04/24/92	7	9	8.04	9.7	126	12.2	2.7	42
	05/12/92	9	12	7.74	13.7	80	7.7	1.7	26
Flint Creek below Douglas Creek	04/05/92	92	5	7.96	33.8	265	33.1	9.8	123
	04/15/92	70	7	8.42	NM	268	33.0	10.1	124
	04/23/92	72	7	8.02	19.5	282	34.6	10.3	129
	05/03/92	36	14	7.76	NM	277	24.9	6.7	90
	05/11/92	41	12	8.31	7.1	191	24.0	6.2	85
	05/20/92	NA	10	8.30	NM	218	27.0	7.6	99

[1] Abbreviations: NM = not measured; NA = not available (lost data).
[2] Detection limits: calcium and magnesium = 1 mg/L
[3] Flows given are visual estimates; flow conditions too low to permit measurement.

The EPA TR method (Method 200.2) digests a 100 ml aliquot of sample using nitric acid and hydrochloric acid. The sample is evaporated on a hot plate to approximately 20 mls, and then refluxed for 30 minutes. The MT TR method analyzes a field preserved (acidification with nitric acid to pH < 2) sample without further digestion. The strong acid and heat involved in the EPA TR method theoretically recovers a greater fraction of the total metal concentration. Therefore, the use of MT TR metals concentrations, instead of EPA TR metals concentrations, to evaluate ambient water quality criteria would be conservative.

MT TR metals concentrations were analyzed every other sampling run. A total of 45 samples (ambient samples not including field QC samples) were analyzed for both EPA TR and MT TR cadmium, copper, lead, and zinc. Of 180 comparisons (4 metals per sample), only 9 (5%) comparisons resulted in a higher MT TR concentration. In four of these nine, the difference was no greater than the detection limit (1 ppb). The other five samples were collected from Silver Bow Creek and had high concentrations of copper and zinc (greater than 100 ppb). MT TR concentrations were generally about 10% higher than their respective EPA TR concentrations.

The data indicate that the EPA TR method results in higher metals concentrations than the MT TR method. Therefore, the use of existing data expressed as MT TR recoverable metals concentrations to evaluate ambient water quality criteria would be conservative.

4.4   Comparison of EPA TR and Dissolved Metals Concentrations

A comparison of dissolved metals concentrations to total recoverable concentrations is useful in detecting or confirming contamination. Dissolved concentrations are expected to be no greater than total recoverable concentrations, because the filtration process removes organic and inorganic material greater than $0.45\mu m$ from the sample prior to analysis. An examination of the data presented in Tables A-1 through A-3 shows only four instances where dissolved concentrations exceed total recoverable concentrations. In all cases, the dissolved concentration was approximately twice the total recoverable concentration. Although contamination of the dissolved sample is always considered in such cases, part of the difference may be artifacts of sample processing (splitting and digesting of the total recoverable sample) and analysis.

4.5   Comparison of Composite and Grab Sampling Methods

A total of seven paired grab/composite samples were collected for EPA TR and dissolved metals analysis. Four of these were also analyzed for MT TR metals. Metals concentrations in composite and grab samples are presented in Table A-7. The RPDs of

Table A-7
Comparison of Grab and Composite Samples
(concentrations in ppb)[1,2]

Date - Sample	TSS (mg/l)	SC (umhos at 25°C)	Cadmium EPA TR	Cadmium MT TR	Cadmium Diss	Copper EPA TR	Copper MT TR	Copper Diss	Lead EPA TR	Lead MT TR	Lead Diss	Zinc EPA TR	Zinc MT TR	Zinc Diss
04/05/92 - Comp	24.4	402	ND	ND	ND	45	15	2	7	3	ND	85	24	6
04/05/92 - Grab	28.7	348	ND	ND	ND	18	16	1	4	3	ND	48	24	9
04/05/92 - Comp	16.2	551	0.3	ND	ND	37	35	10	3	3	ND	52	41	11
04/05/92 - Grab	21.2	550	0.2	ND	ND	42	38	10	4	3	ND	55	43	8
04/15/92 - Comp	8.0	574	0.2	NM	ND	26	NM	7	2	NM	ND	30	NM	11
04/15/92 - Grab	8.7	559	ND	NM	ND	28	NM	8	3	NM	ND	29	NM	14
04/24/92 - Comp	88.2	556	ND	ND	ND	2	1	ND	2	2	ND	8	7	ND
04/24/92 - Grab	42.8	551	ND	ND	ND	2	2	ND	2	2	ND	7	8	ND
05/03/92 - Comp	NM	277	ND	NM	ND	2	NM	ND	5	NM	ND	13	NM	ND
05/03/92 - Grab	NM	225	ND	NM	ND	2	NM	ND	5	NM	ND	14	NM	1
05/11/92 - Comp	7.1	191	ND	ND	ND	3	3	1	4	4	ND	11	9	ND
05/11/92 - Grab	8.1	192	ND	ND	ND	2	1	ND	4	4	ND	11	11	ND
05/20/92 - Comp	NM	606	ND	NM	ND	4	NM	3	ND	NM	ND	5	NM	4
05/20/92 - Grab	NM	598	ND	NM	ND	5	NM	2	ND	NM	ND	5	NM	2

[1] Abbreviations: NM (not measured), ND (concentration below detection limits).
[2] Detection limits: cadmium (0.2 ppb); copper, lead and zinc (1 ppb).

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composite and grab samples are summarized in Table A-8 (a negative RPD indicates that the grab sample concentration was lower than the composite sample concentration). The 20% RPD is generally not applied to concentrations less than or equal to five times the analytical detection limit (0.2 $\mu g/l$ cadmium; 1 $\mu g/l$ copper, lead, zinc) because of potentially large RPDs resulting from normal analytical variability at low analyte concentrations, and by rounding of data. With these considerations, most of the grab and composite samples fall within the 20% RPD threshold (or were not calculable due to concentrations below the detection limit). A total of 6 EPA TR and dissolved comparisons (of a total 56) were outside the 20% RPD threshold. Four of these occurred in one sample (collection date 04/05/92), with RPDs ranging from 40% for dissolved zinc to 86% for EPA TR copper.

5.0   QUALITY ASSURANCE/QUALITY CONTROL

An evaluation of quality control includes an assessment of field splits, field duplicates, and field blanks (filter, trip, and decontamination).

There are no EPA control limits nor corrective actions for field QC statistics (PTI, 1989). EPA considers field QC as "useful in assessing a laboratory's performance independent of sample or method problems," and primarily "useful as supporting evidence in the overall assessment" of a data set or sampling event (PTI, 1989). It further concludes that field QC "is not the basis of accepting or rejecting data, but rather...additional evidence in support of these conclusions arrived at by a review of the total package." Therefore, except in the case of gross errors, poor performance on field QC samples does not result in invalidating data.

Field splits and duplicates are useful in evaluating the replicability of field sampling and subsampling protocols. RPDs were calculated for split and duplicate samples; the same threshold (20%) used for laboratory splits and duplicates, was applied. These results are summarized in Tables A-9 through A-12.

Field blanks are useful in assessing potential sources of sample contamination. Filter blanks were used to assess the thoroughness of equipment cleaning and preparation, and potential contamination from the filters and field filtering procedure. Trip blanks measure potential sample contamination from the sample bottle, reagent water or preservative, or contamination from preparing, preserving, handling or transporting sample containers from the laboratory to the field and back. Decontamination blanks measure the effectiveness of sampling equipment decontamination. A control limit of less than 2X the instrument detection limit (IDL) may be applied to field blanks, as this is the control limit established by the QAPP for initial calibration and continuing calibration blanks. Results of filter, trip, and decontamination blanks are summarized in Tables A-13 through A-15.

Table A-8

Relative Percent Difference of Grab and Composite Samples

Date	TSS	SC	Cadmium			Copper			Lead			Zinc		
			EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
04/05/92	16	-14	NC	NC	NC	-86	6	NC	-55	NC	NC	-56	0	40
04/05/92	27	0	NC	NC	NC	13	8	0	NC	NC	NC	6	5	-32
04/15/92	8	-3	NC	NM	NC	7	NM	13	NC	NM	NC	-3	NM	24
04/24/92	-69	-1	NC	NC	NC	NC	NC	NC	NC	NC	NC	-13	13	NC
05/03/92	NM	-21	NC	NM	NC	NC	NM	NC	NC	NM	NC	14	NM	NC
05/11/92	13	0	NC	NC	NC	NC	NC	NC	NC	NC	NC	0	20	NC
05/20/92	NM	-1	NC	NM	NC	NC	NM	NC	NC	NM	NC	NC	NM	NC

Abbreviations: NM (not measured); NC (not calculable -- both concentrations less than 5X the instrument detection limit (IDL); difference in concentrations not greater than 2X IDL); NC* (not calculable; both concentrations less than 5X the instrument detection limit (IDL); difference in concentrations greater than 2X IDL).

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Table A-9
Results of Field Split Sample Analyses
(concentrations in ppb)[1,2]

Date - Sample	TSS (mg/l)	SC (umhos at 25° C)	Cadmium			Copper			Lead			Zinc		
			EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
04/15/92 - Sample	7.9	553	ND	NM	NM	24	NM	NM	2	NM	NM	28	NM	NM
04/15/92 - Split	8.4	564	ND	NM	NM	25	NM	NM	3	NM	NM	31	NM	NM
04/24/92 - Sample	88.7	548	ND	ND	NM	8	1	NM	2	2	NM	11	5	NM
04/24/92 - Split	86.7	550	ND	ND	NM	2	2	NM	5	3	NM	8	7	NM
05/03/92 - Sample	NM	204	ND	NM	NM	3	NM	NM	6	NM	NM	15	NM	1
05/03/92 - Split	NM	203	ND	ND	NM	3	NM	NM	5	NM	NM	17	NM	ND
05/11/92 - Sample	5.9	194	ND	ND	NM	2	1	NM	4	3	NM	10	9	NM
05/11/92 - Split	6.9	194	ND	ND	NM	1	1	NM	3	3	NM	10	9	NM
05/20/92 - Sample	NM	602	ND	NM	NM	5	NM	NM	ND	NM	NM	6	NM	NM
05/20/92 - Split	NM	601	ND	NM	NM	4	NM	NM	ND	NM	NM	5	NM	NM

[1] Abbreviations: NM (not measured), ND (concentration below detection limits).
[2] Detection limits: cadmium (0.2 ppb); copper, lead and zinc (1 ppb).

Table A-10

Relative Percent Difference of Split Samples

Date	TSS	SC	Cadmium			Copper			Lead			Zinc		
			EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
04/15/92	6	2	NC	NM	NM	4	NM	NM	NC	NM	NM	7	NM	NM
04/24/92	2	0	NC	NC	NM	120	NC	NM	NC*	NC	NM	32	33	NM
05/03/92	NM	0	NC	NM	NM	NC	NM	NM	18	NM	NM	6	NM	NC
05/11/92	16	0	NC	NC	NM	NC	NC	NM	NC	NC	NM	0	NC	NM
05/20/92	NM	0	NC	NM	NM	NC	NM	NM	NC	NM	NM	18	NM	NM

Abbreviations: NM (not measured); NC (not calculable: both concentrations less than 5X the instrument detection limit (IDL); difference in concentrations not greater than 2X IDL); NC* (not calculable; both concentrations less than 5X the instrument detection limit (IDL); difference in concentrations greater than 2X IDL).

Table A-11
Results of Field Duplicate Sample Analyses
(concentrations in ppb)[1,2]

Date - Sample	TSS (mg/l)	SC (umhos at 25°C)	Cadmium			Copper			Lead			Zinc		
			EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
04/06/92 - Sample	4.4	121	ND	ND	ND	7	5	5	1	ND	ND	20	20	16
04/06/92 - Duplicate	4.4	131	ND	ND	ND	6	3	4	1	ND	ND	27	14	19
04/15/92 - Sample	8.0	574	0.2	NM	ND	26	NM	7	2	NM	ND	30	NM	11
04/15/92 - Duplicate	7.9	553	ND	NM	ND	24	NM	ND	3	NM	ND	29	NM	12
04/24/92 - Sample	88.2	556	ND	ND	NM	2	1	NM	2	2	NM	8	7	NM
04/24/92 - Duplicate	88.7	548	ND	ND	NM	8	1	NM	2	2	NM	11	5	NM
05/03/92 - Sample	NM	277	ND	NM	ND	2	NM	ND	5	NM	ND	13	NM	ND
05/03/92 - Duplicate	NM	204	ND	NM	ND	3	NM	ND	6	NM	ND	15	NM	1
05/11/92 - Sample	7.1	191	ND	ND	ND	3	3	1	4	4	ND	11	9	ND
05/11/92 - Duplicate	5.9	194	ND	ND	ND	ND	1	ND	4	3	ND	10	9	ND
05/20/92 - Sample	NM	606	ND	NM	ND	4	NM	3	ND	NM	ND	5	NM	4
05/20/92 - Duplicate	NM	602	ND	NM	ND	5	NM	3	ND	NM	ND	6	NM	2
05/21/92 - Sample	5.8	199	0.3	NM	ND	8	NM	3	ND	NM	ND	6	NM	1
05/21/92 - Duplicate	5.5	199	ND	NM	ND	7	NM	3	1	NM	ND	5	NM	ND

[1] Abbreviations: NM (not measured), ND (concentration below detection limits).
[2] Detection limits: Cadmium (0.2 ppb); copper, lead and zinc (1 ppb).

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Table A-12

Relative Percent Difference of Field Duplicate Samples

Date	Cadmium			Copper			Lead			Zinc		
	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss	EPA TR	MT TR	Diss
04/06/92	NC	NC	NC	15	NC	NC	NC	NC	NC	30	35	17
04/15/92	NC	NM	NC	8	NM	150	NC	NM	NC	3	NM	9
04/24/92	NC	NC	NM	120	NC	NM	NC	NC	NM	32	33	NM
05/03/92	NC	NM	NC	NC	NM	NC	18	NM	NC	14	NM	NC
05/11/92	NC	NC	NC	NC*	NC	NC	NC	NC	NC	10	0	NC
05/20/92	NC	NM	NC	NC	NM	NC	NC	NM	NC	18	NM	NC
05/21/92	NC	NM	NC	13	NM	NC	NC	NM	NC	18	NM	NC

Abbreviations: NM (not measured); NC (not calculable: both concentrations less than 5X the instrument detection limit (IDL); difference in concentrations not greater than 2X IDL); NC* (not calculable; both concentrations less than 5X the instrument detection limit (IDL); difference in concentrations greater than 2X IDL.

Table A-13
Results of Filter Blank Analyses, Dissolved Concentrations[1,2]

Date	Ca (mg/l)	Mg (mg/l)	Cadmium (μg/l)	Copper (μg/l)	Lead (μg/l)	Zinc (μg/l)
04/06/92	ND	ND	ND	ND	ND	4
04/15/92	ND	ND	ND	ND	ND	3
04/24/92	ND	ND	ND	ND	ND	ND
05/03/92	ND	ND	ND	ND	ND	ND
05/11/92	ND	ND	ND	ND	ND	ND
05/20/92	ND	ND	ND	ND	ND	ND

[1] Abbreviations: ND (concentration below detection limits).
[2] Detection limits: Cadmium (0.2 μg/l); copper, lead and zinc (1 μg/l); calcium and magnesium (1 mg/l).

Table A-14
Results of Trip Blank Analyses[1,2]

Date	TSS (mg/l)	SC (umhos at 25° C)	Cadmium (μg/l)		Copper (μg/l)		Lead (μg/l)		Zinc (μg/l)	
			EPA TR	MT TR	EPA TR	MT TR	EPA TR	MT TR	EPA TR	MT TR
04/06/92	NM	NM	ND	ND	ND	ND	1.3	ND	18.5	2.0
04/15/92	ND	26	ND	NM	ND	ND	ND	NM	1.5	NM
04/24/92	NM	NM	ND	ND	ND	ND	ND	ND	3.0	ND
05/03/92	ND	30	ND	NM	ND	ND	ND	NM	1.5	NM
05/11/92	ND	5	ND	ND	ND	ND	ND	ND	ND	ND
05/20/92	ND	3	ND	NM	1.8	NM	ND	NM	3.5	NM

[1] Abbreviations: NM (not measured), ND (concentration below detection limits), EPA TR (EPA total recoverable), MT TR (Montana total recoverable).
[2] Detection limits: Cadmium (0.2 μg/l); copper, lead and zinc (1 μg/l); TSS (1 mg/l).

Table A-15
Results of Decontamination Sample Analyses, EPA Total Recoverable (EPA TR)
and Montana Total Recoverable (MT TR)
(concentrations in ppb)[1,2]

Date	Cadmium		Copper		Lead		Zinc	
	EPA TR	MT TR	EPA TR	MT TR	EPA TR	MT TR	EPA TR	MT TR
04/06/92	ND	ND	7.4	5.0	2.0	1.1	14.5	9.0
04/15/92	ND	NM	1.1	NM	1.3	NM	3.0	NM
04/24/92	ND	ND	1.8	1.8	ND	9.3	ND	ND
05/03/92	ND	NM	ND	NM	ND	NM	2.8	NM
05/11/92	ND	ND	ND	ND	ND	ND	1.5	ND
05/20/92	ND	NM	ND	NM	ND	NM	2.0	NM

[1] Abbreviations: NM (not measured), ND (concentration below detection limits).
[2] Detection limits: Cadmium (0.2 ppb); copper, lead and zinc (1 ppb).

Filter blanks (Table A-13) generally showed undetectable concentrations of the constituents of interest. Two samples contained zinc concentrations (4 and 3 ppb) on the first two sampling dates.

Trip blanks (Table A-14) generally showed undetectable concentrations of cadmium, copper, and lead. Two measurable copper and lead concentrations were less than 2X the IDL. EPA TR zinc concentrations were measurable in five of the six filter blanks, three of which were greater than 2X IDL. The largest concentration (19 ppb) had a corresponding MT TR zinc concentration of 2 ppb. This indicates that the high zinc concentration may have resulted from contamination of the sample during laboratory processing.

Decontamination blanks (Table A-15) generally show undetectable or slightly detectable concentrations of most constituents. The blank collected on 4/6/92 contained copper and zinc concentrations exceeding 2X IDL. The blank collected on 4/24/92 contained 9 ppb MT TR lead. The lead result is inconclusive because the EPA TR concentration was undetectable.

6.0 REFERENCE

PTI. 1989. Silver Bow Creek Water Quality and Sediment Sampling Data (Draft). Prepared by PTI Environmental Services for Parcel, Mauro, Hultin, & Spaanstra, Denver, CO.

AQUATIC RESOURCES INJURY REPORT

APPENDIX B

Acute Toxicity in Pulse Events:
Relative Sensitivity of Brown and Rainbow Trout to Pulses of Metals
Typical of the Clark Fork River

Prepared by:

Harold Bergman, University of Wyoming

AQUATIC RESOURCES INJURY REPORT

APPENDIX C

Influence of Acclimation/Adaptation on Toxicity:
Differential Tolerance and Resistance of Brown and Rainbow Trout to Water-borne Metal
Concentrations Typical of the Clark Fork River

Prepared by:

Harold Bergman, University of Wyoming

FINAL REPORT

Acute Toxicity in Pulse Events:

Relative Sensitivity of Brown and Rainbow Trout to Pulses of Metals Typical of the Clark Fork River

Research Report on Injury Determination

Fishery Protocol #3

Assessment Plan, Part I

Clark Fork River Basin NPL Sites, Montana

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Submitted by:

Harold L. Bergman

Red Buttes Environmental Biology Laboratory

University of Wyoming

Laramie, Wyoming 82071

May 1993

INTRODUCTION

Copper, Zn, Pb, and Cd are the hazardous substances most frequently elevated above water quality criteria in the Clark Fork River (USEPA 1987; USGS 1989; Lambing 1991; Moore et al. 1991). These metals are classified as "very toxic and relatively accessible" and are among the metals of highest concern for toxic effects on fish (Förstner and Wittman 1983; Baudo 1987; Heath 1987). Once these metals enter aquatic systems they persist in various chemical forms and may become available to biota under appropriate environmental conditions (Baudo 1987). For example, metals may reach acutely lethal concentrations following rainstorms that produce acidic runoff originating from streamside tailings. Acidic runoff can also mobilize metals from the sediments (Turnpenny et al. 1987; Brooks and Moore 1989). As recently as July 1989, for instance, 5,300 fish (including 2,300 brown trout) were killed in the upper Clark Fork River under these circumstances (G.R. Phillips, written commun. 1989, cited in Nimick and Moore 1991). In addition, physical conditions such as ice breakup and spring runoff promote resuspension of metals bearing substrates into the water column (USGS 1989).

The principal objective of this study was to determine the acute toxicity of pulsed concentrations of metal mixtures that occur in the Clark Fork River to brown and rainbow trout. This objective included an evaluation of the relative sensitivity to a pulsed metal exposure for (1) brown trout fry from the Clark Fork River and from a hatchery stock and rainbow trout from a hatchery stock and, (2) early lifestage and juvenile brown trout and rainbow trout to a pulsed metal exposure.

METHODS

Procedures employed for the experiments were those specified under Research Plans, Section 7.4.4, in the Assessment Plan: Part I, Clark Fork River NPL Sites, Montana (Montana 1992; hereafter referred to as the Assessment Plan) except where noted.

Experimental Fish

Hatchery rainbow trout and brown trout were obtained as eyed embryos in the winter of 1991 from Dubois Fish Hatchery, Wyoming. Juvenile rainbow trout and brown trout were obtained during the summer of 1992 from Dan Speas Fish Hatchery and from the Dubois Fish Hatchery, respectively; both hatcheries are operated by the Wyoming Game and Fish Department. In the fall of 1991, wild-caught brown trout from the upper Clark Fork River (Warm Springs area) were spawned and the eggs fertilized in a 1:1 (male to female) ratio by fisheries biologists of the Montana Department of Fish, Wildlife and Parks [Note: brown trout from the Big Hole River were not included in these experiments, although originally included in the Assessment Plan]. Fertilized eggs were held in Clark Fork water and subsequently were transported to the Red Buttes Environmental Biology Laboratory, University of Wyoming. The eggs were acclimated to laboratory culture conditions in Heath Incubators and upon hatching transferred to holding tanks, maintained, and handled according to the Assessment Plan. All eggs and fry were treated with 100 mg/L Betadine, 6 mg/L Chloramine T, and 2% NaCl (Doug Mitchum, Fish Pathologist, Wyoming Game and Fish, pers. comm.).

Following swim-up stage and throughout the holding periods, fry (mean weight 0.184 g; mean length 28.6 mm) were fed 5% body weight of Silver Cup Starter Chow, brine shrimp, Romet 30 treated chow, and eventually remained on Biodiet chow. Juveniles (mean weight 21.230 g; mean length 126.5 mm) were fed 2% body weight of vitamin-fortified commercial trout diet [Note: this is the recommended feeding ration specified for juvenile salmonids at 10 °C (Piper et al. 1986) and was used rather than the 5% of body weight per day specified in the Assessment Plan]. Fish were acclimated to control-water conditions

specified for each respective test for several weeks and during this period they were monitored daily and remained in good condition free of disease. Thirty-eight hours prior to each experiment, fish were transferred to exposure chambers and deprived of food. Photoperiod was maintained to simulate natural-light cycles throughout the study period.

Exposure Water

Test and control waters were formulated by continuously mixing well water and deionized water to simulate various conditions in the Clark Fork River. Water hardness levels above 400 mg/L $CaCO_3$ were achieved by metering a $CaSO_4$ stock solution into the formulated waters.

Test water was identical to the control water, but contained one of four dilutions of an 8P metals mixture during the pulsed exposures, where 1P = 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. The metal concentrations in the 1P reference mixture are a conservative representation of the metal concentrations that have been measured during episodic (pulse) events and during "redwater" discharge events documented in the Clark Fork River from as early as 1960 and before and as recently as 1991 (see Table 1 and Surface Water Resources Chapter). The specific metal concentrations (in μg/L) selected for Zn, Cu and Cd in the 1P reference metal mixture, 230 Zn, 120 Cu and 2.0 Cd, were the dissolved concentrations actually measured in the Clark Fork River at Deer Lodge during the documented fish kill on July 12, 1989 (Table 1); because dissolved Pb concentrations were below detection limits for this particular pulse sample, the Pb concentration for the 1P reference pulse was set at 3.2 μg/L, which is the chronic water quality criterion for a water hardness of 100 mg/L $CaCO_3$ (USEPA 1987). This set of metal concentrations for the reference (1P) pulse metal mixture used in laboratory experiments is conservative, given the remarkably high total recoverable metal concentrations measured at different locations and times during storm pulse or "redwater" discharge events documented on the upper Clark Fork River (see Table 1 for a summary). All exposure concentrations used for each metal are within the range of concentrations observed in the Clark Fork River as described in the Surface

Experimental Design: Fry Pulsed Exposures (Tests I - VI)

Hatchery brown trout and hatchery rainbow trout fry were used in each of the six experiments; Clark Fork brown trout fry were used in two of the experiments (Table 2). Fish were acclimated for a minimum of one month to the control water conditions employed during a given experiment (e.g., hardness, 100 mg/L $CaCO_3$; alkalinity, 80 - 110 mg/L $CaCO_3$; pH, 7.2 - 8.0; temperature, 10 °C). Position for the various species/strains used and replicate-treatment blocks were randomly assigned. Individuals of each species were sequentially transferred in groups of two from holding chambers to exposure or control chambers, thus randomizing the allocation of individuals to exposures. Ten individuals were used per exposure concentration and control within each replicate block unless otherwise indicated.

Mortality (cessation of opercular movement) was monitored every twenty to forty minutes during the pulsed exposure and twice daily for the 96 hours that followed. Dead fish were removed immediately and frozen for later determination of length, weight, etc.

During the pulsed exposure, each exposure dilution was increased steadily for 1 hour, held constant for 6 hours, and returned to control conditions over 1 hour (1,6,1-hour pulse) after which water chemistry was kept constant (Note: the pulsed exposure period and post-pulse observation period will be referred to as 8 + 96-hr). Additionally, hardness, alkalinity, and pH either remained constant, were depressed, or were increased during the pulse and changed in the same time course as metal concentrations (Table 2).

Experimental Design: Juvenile and Fry Pulsed Exposure (Tests VII and VIII)

Fish were acclimated for a minimum of one month to control water conditions (hardness, 200 mg/L $CaCO_3$; alkalinity, 180 - 200 mg/L $CaCO_3$; pH, 7.2 - 8.0; temperature, 10 °C). Each species (hatchery brown trout, fry and juveniles, and hatchery rainbow trout, fry and juveniles) used in the first experiment (Test VII) was randomly assigned to a set of exposure tanks to not bias any species due to position; this assignment was reversed for a

second-duplicate experiment (Test VIII). Individuals of each species and life stage were sequentially transferred in groups of two from holding tanks to exposure or control tanks, thus randomizing the allocation of individuals to treatments. Ten individuals of each species and life stage were used for each treatment replicate.

Mortality (cessation of opercular movement) was monitored every twenty to forty minutes during the pulsed exposure and twice daily for the 96 hours that followed. Dead fish were removed immediately and frozen for later determination of length, weight, etc.

During the pulsed exposure, each exposure dilution was increased steadily for approximately 2 hours, held constant for 4 hours, and returned to control water conditions over 2 hours (2,4,2-hour pulse) after which water chemistry was kept constant (Note: the pulsed exposure period and post-pulse observation period will be referred to as 8 + 96-hr). Additionally, hardness and pH were depressed and then increased (200 to 100 to 200 mg/L $CaCO_3$ and pH 7.8 to 4.5 to 7.8) during the pulse and changed in the same time course as metal concentrations.

Chemical Analyses of Water

Test and control waters were analyzed for metals, hardness, alkalinity, pH, and dissolved oxygen at specified intervals prior to and during the pulsed exposure and each day following.

Statistical Analyses

To evaluate relative sensitivity of the test fish to the metals mixture, LC50 values and 95% confidence intervals were computed using the Spearman-Karber method (Hamilton et al. 1977). LC50 values were computed using average exposure concentrations of Zn (in μg/L) analyzed by atomic absorption spectroscopy; Zn was used as the representative metal-species because of its relative abundance in the Clark Fork River metal mixture and because of the greater precision with which it was measured analytically due to its higher relative abundance in the metal mixture. The LC50 estimates were determined to be significantly different if the

95% confidence intervals did not overlap. This is analogous to a one-tailed test of significant differences at $\alpha = 0.05$ with overall protection of $(0.95)^n$, where n is the number of species/strain being compared.

In addition, relative sensitivity of the test fish to the exposure concentrations was evaluated using a two-way Analysis of Variance (ANOVA) to investigate the effects of the metals concentrations and the blocks (replicates) on the mean proportion surviving the pulsed-exposures and the 96 hour post-pulse period for each species and each test (Tests I-VI experimental design = completely randomized block; Tests VII and VIII experimental design = completely randomized block with blocks separated by time). There were five levels for the metals-concentrations factor (8P, 4P, 2P, 1P, and control), with each level containing up to eight blocks (replicates), depending on the number of replicates employed. The response variable used in the analysis was the $\arcsin\sqrt{p}$ transformed value (Sokal and Rohlf 1981), where p was the proportion of fish surviving. The block x treatment interaction mean square was used to test for significant treatment effects at the $\alpha = 0.10$ level. Dunnett's multiple comparison procedure (Zar 1984) was conducted to compare survival from each exposure concentration to the corresponding control survival. One-tailed p-values from Dunnett's procedure below the $\alpha = 0.05$ level were judged to be significant. The Dunnett's comparisons determined the highest concentration of metals (P units) having no statistical difference in survival from the control (NOEC, no observed effect concentration) and the lowest concentration of metals (P units) having a statistically reduced survival from the control (LOEC, lowest observed effect concentration) (Gelber et al. 1985).

RESULTS

Water Chemistry

Measured hardness, alkalinity, and pH did not deviate from the nominal values by more than 26% in all tests for all characteristics (Appendix Table 1). Dissolved oxygen was greater than 75% saturation and temperature remained at 10.0 ± 1.5 °C in all exposure chambers for all tests (Appendix Table 2).

Maximum average Zn concentrations were 72 - 99% of the nominal concentrations. Zinc concentration averages and standard deviations for the different exposure dilutions from the pre-pulse, mid-pulse (average maximum concentration), and post-pulse periods are shown in Appendix Table 3. Copper, Pb and Cd average maximum concentrations (i.e., from the mid-pulse period) and standard deviations for the different exposure dilutions are shown in Appendix Table 4.

Fry Pulsed Exposures

Survival was between 90 and 100% for all control fish during the pulse and post-pulse periods (Appendix Table 6 and Appendix Figures 1a-1f). Mortalities were observed as early as 2 hours into the pulse in the highest exposure concentration and cumulative survivals were typically reduced to 0% for hatchery brown and rainbow trout within 24 hours following the 8 hour pulsed exposure as shown in the survival plots for each test (Appendix Figures 1a-1f).

Hatchery rainbow trout were more tolerant (i.e., significantly higher LC50, $\alpha = 0.05$; Table 3 and Figure 1) than hatchery brown trout under constant hardness and pH, but under depressed hardness and depressed pH, hatchery rainbow trout were less tolerant than hatchery and Clark Fork brown trout (i.e., significantly lower LC50, $\alpha = 0.05$; Table 3 and Figure 1). In all tests having depressed hardnesses and depressed hardness and pH, hatchery rainbow trout had lower LC50s than their LC50 under constant hardness and pH (Table 3 and Figure 1). Additionally, hatchery rainbow trout were less tolerant (lower LC50) in tests where the hardness, as ppm $CaCO_3$, was initially 100 and depressed to 50 than in tests where the

hardness was initially 200 and depressed to 100. This suggests that the lower initial hardness level (i.e., hardness 100 compared to 200 ppm) reduced survival in the hatchery rainbow trout moreso than the degree of change in hardness level. A similar trend was found for hatchery brown trout, although no significant difference was observed in the LC50 from Test III (hardness depressed from 100 to 50) compared to Test V (hardness depressed from 200 to 100). Tolerance was clearly greater (higher LC50s) for both hatchery rainbow and brown trout with elevated hardness (from 200 to 420 mg/L $CaCO_3$, Test VI) than in the other pulse conditions.

In the two tests that included Clark Fork brown trout (Tests IV and V), they were slightly more tolerant than the hatchery species/strains, as shown by higher survival rates in these tests (Appendix Figures 1d and 1e). The LC50 values for Clark Fork brown trout were similar from Tests IV and V, yet hatchery brown trout had a significantly lower LC50 in Test V than in Test IV and had a significantly lower LC50 than Clark Fork browns in Test V (Table 3 and Figure 1). The LC50s for Clark Fork and hatchery brown trout from these two tests (Tests IV and V), having equivalent hardnesses, alkalinities, and pHs, suggest that Clark Fork brown trout respond similarly or are slightly more tolerant to the exposure mixture than hatchery brown trout.

In addition to the above analysis, survival was evaluated using Dunnett's comparisons of treatment survival to control survival to determine the lowest observed effect concentrations (LOEC) and the no observed effect concentrations (NOEC). Metal concentrations as low as 1P, 2P, and 4P significantly reduced survival in hatchery rainbow trout, hatchery brown trout, and Clark Fork brown trout, respectively, following eight hour pulsed exposures and 96 hour post-pulse observation periods when water hardness or pH/hardness were depressed concurrently with the metals exposure (Table 4).

In Test I (constant hardness & pH), the LOEC was 4P for hatchery brown trout and hatchery rainbow trout (Table 4). However, under Test I conditions hatchery rainbow trout had slightly higher survival proportions than hatchery brown trout (Appendix Tables 5 and 6).

In Test II (depressed hardness, constant pH), the LOEC was 2P for hatchery brown trout and 1P for hatchery rainbow trout (Table 4). In other words, under Test II conditions hatchery brown trout had higher survival proportions than hatchery rainbow trout (Appendix Tables 5 and 6).

In Test III (depressed hardness & pH), the LOEC was 2P for hatchery brown trout and 1P for hatchery rainbow trout (Table 4). Again, under Test III conditions hatchery brown trout had higher survival proportions than hatchery rainbow trout (Appendix Tables 5 and 6).

In Test IV (highly depressed hardness & depressed pH), the LOEC was 4P for hatchery brown trout and Clark Fork brown trout and 1P for hatchery rainbow trout (Table 4). Under Test IV conditions, hatchery brown trout had similar survival proportions to Clark Fork brown trout, which had higher survival proportions than hatchery rainbow trout (Appendix Tables 5 and 6).

In Test V (highly depressed hardness & depressed pH), the LOEC was 4P for hatchery brown trout, 2P for hatchery rainbow trout, and 8P for Clark Fork brown trout (Table 4). Under Test V conditions, Clark Fork brown trout had higher survival proportions than hatchery brown trout, which had higher survival proportions than hatchery rainbow trout (Appendix Tables 5 and 6).

In Test VI (highly elevated hardness & depressed pH), the LOEC was >8P for hatchery brown trout and 8P for hatchery rainbow trout (Table 4). Under Test VI conditions, hatchery brown trout had higher survival proportions than hatchery rainbow trout (Appendix Tables 5 and 6).

Juvenile and Fry Pulsed Exposure

Survival was 100% for all control fish during the pulse and post-pulse periods (Tests VII and VIII; Appendix Table 7 and Appendix Figures 2a and 2b) for juvenile and fry hatchery brown trout and hatchery rainbow trout. For each species/lifestage, there was little difference in survival rates between Tests VII and VIII (Appendix Figures 2a and 2b), therefore these tests were treated as replicates in the LC50 calculations and in the Dunnett's

comparisons for survival analysis. In the highest exposure concentration, mortalities were observed as early as 3 hours into the pulse for both hatchery rainbow trout juveniles and fry, and cumulative survivals were reduced to 0% during the pulse and within 24 hours following the pulse for hatchery rainbow trout juveniles and fry, respectively (Appendix Figures 2a and 2b). In the highest exposure concentration, mortalities were observed by 7 hours into the pulse for both hatchery rainbow trout juveniles and fry, and cumulative survivals were reduced to 60% and 15% at the end of the post-pulse period (96 hours) for hatchery rainbow trout juveniles and fry, respectively (Appendix Figures 2a and 2b).

Hatchery rainbow trout juveniles and fry were similar in sensitivity to the pulsed exposure (i.e., LC50 estimates were not significantly different; Table 5). Whereas hatchery brown trout juveniles were less sensitive than hatchery brown trout fry, yet because confidence limits could not be calculated for hatchery brown trout juveniles a stated statistical significance could not be assigned (Table 5).

The LOEC was 4P for hatchery brown trout fry and hatchery rainbow trout fry and juveniles and was 8P for hatchery brown trout juveniles (Table 6). Mean survival proportions were lowest for hatchery rainbow trout juveniles followed by hatchery rainbow trout fry, hatchery brown trout fry and hatchery brown trout juveniles (Appendix Table 7). Thus, both lifestages of the hatchery rainbow trout appeared more sensitive than either lifestage of the hatchery brown trout.

DISCUSSION

Acute Lethality of Clark Fork Metal Concentrations

Survival of both brown and rainbow trout was significantly reduced in this laboratory study when trout were episodically exposed to a metal mixture containing Zn, Cu, Pb and Cd, within the range of concentrations and ratios observed during fish kill events in the Clark Fork River. From the eight hour exposures (during which maximum concentrations were present for six hours or less), metal concentrations as low as 1P (2P for hatchery brown trout) adversely affected fry survival.

The episodes of fish kills in the Clark Fork River during "redwater" and storm events at which elevated concentrations of Zn, Cu, Pb and Cd have been observed (Table 1) suggests that the metal concentrations are sufficiently elevated and sustained during these events to induce mortality in resident fish populations. Laboratory studies cannot represent the full range of toxic conditions that may occur during fish kills. For example, the duration of exposure in the Clark Fork River may, at times, be either shorter or longer than the 8-hour time period used in representing pulse events, the water temperature in the laboratory was held constant at 10 °C, and the laboratory results are reported here as discrete time (i.e., 8 + 96-hour) median lethal concentrations or LC50's, to facilitate the most robust statistical and toxicological comparison of species/strain sensitivities, whereas metal concentrations that would cause a substantial fish kill in the field could be as low as the "LC1" or "LC10" concentration (where 1% and 10%, respectively, of a population would be killed). Overall, the results reported here for controlled laboratory experiments on the episodic/acute lethality of metal mixtures, at concentrations that have been documented at equal or higher concentrations during pulse or "redwater" discharge events in the Clark Fork River, clearly support a conclusion that fish kills associated with these events were caused by toxic metal concentrations. The results in this study support the conclusion that a short-term pulsed exposure to metals and variable water quality characteristics typical of the Clark Fork River will induce mortality in brown and rainbow trout.

The primary objectives of this study were to determine (1) the differential sensitivity of early lifestage hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout to episodic metals exposure typical of the Clark Fork River, and (2) the differential sensitivity of early lifestage and juvenile lifestage of both hatchery brown and rainbow trout to episodic metals exposure typical of the Clark Fork River. To address these objectives we calculated the relative sensitivity, as LC50s, of the different species/strains and lifestages when episodically exposed to a metals mixture typical of the Clark Fork River. In addition to these studies, companion studies on brown and rainbow trout, conducted under the other fishery protocols in the Assessment Plan, produced information on relative sensitivity under different conditions. These other studies included influence of acclimation/adaptation on acute toxicity (Fishery Protocol #5), chronic toxicity from food-chain exposures (Fishery Protocol #1), chronic toxicity due to physiological impairment from the food-chain exposures and from the field (Fishery Protocol #2), and chronic (low metal concentration) effects on behavioral avoidance responses (Fishery Protocol #4). Table 7 summarizes the relative sensitivities for brown and rainbow trout to the various acute and chronic exposures used to determine the extent of injury to Clark Fork fisheries resources. From the different measured responses it appears that brown and rainbow trout do not have equivalent sensitivities to the various exposure conditions (Table 7).

Under acutely lethal metal pulse conditions used in the present study the relative sensitivity of the three species/strains is altered dramatically depending on the water quality conditions that accompany the pulsed metal exposure. If water hardness and pH are held constant during the metal pulse, brown trout are more sensitive than rainbow trout. But under conditions that are more typical of storm pulse chemistry in the Clark Fork River (e.g., depressed hardness and pH), the relative sensitivity to the metal pulse is reversed, with rainbow trout being the most sensitive. In the pulsed exposures employing Clark Fork brown trout and using typical storm pulse water chemistry in the Clark Fork River (e.g., depressed

hardness and pH), the Clark Fork brown trout were less sensitive than hatchery rainbow trout and either similar or slightly less sensitive than hatchery brown trout (Table 7).

Similarly, as presented in the companion report on the influence of acclimation and adaptation on toxicity of Clark Fork metal mixtures (Fishery Protocol #5) and summarized in Table 7, the ambient water chemistry conditions affect the relative sensitivities of brown and rainbow trout. Using constant water hardness and pH during the metals exposure and using naive (non-acclimated to metals) fish, brown trout were more sensitive than rainbow trout. But with chronic metal exposures typical of the Clark Fork River, the relative sensitivity of brown and rainbow trout is reversed, with rainbow trout being the most sensitive and Clark Fork brown trout generally the least sensitive to an acutely lethal challenge exposure with a 1P metals mixture. Thus, when acclimated to low-level metal concentrations, Clark Fork brown trout are more resistant to an acutely lethal metal challenge than either hatchery browns or rainbows; and rainbow trout are generally the most sensitive of the tested species/strains under these conditions (Table 7).

The relative sensitivity of rainbow and brown trout to chronic exposures at low metal concentrations was evaluated in the accompanying reports on Fishery Protocols 1, 2 and 4 on food chain, physiological impairment and behavioral avoidance, respectively, and overall conclusions are again summarized in Table 7. Because of reduced feeding activity and, possibly, other causes, rainbow trout are somewhat more sensitive to the adverse effects of dietary metals (from the Clark Fork River) on growth. But in at least three physiological/structural measurements in these experiments (gut impaction, lipid peroxidation and histology; Table 7) brown trout appear to be more sensitive than rainbow trout.

Among all of these laboratory studies on the acute and chronic responses of brown and rainbow trout to Clark Fork metal mixtures, the most marked difference in species sensitivity was observed with behavioral avoidance. As documented in the Fishery Protocol #4 report and summarized in Table 7, rainbow trout were substantially more sensitive than brown trout in avoiding low metal concentrations typical of even base flow conditions in the Clark Fork.

In summary, the relative sensitivity of the three species/strains varies with exposure condition and response measured. But considering the water quality conditions that prevail in the Clark Fork River, continuous low-level, chronic metal concentrations with frequent, short-duration metal excursions that are sufficiently elevated to cause substantial fish kills, rainbow trout appear to be more vulnerable to the direct and indirect effects of the metals than are brown trout. And of the three species/strains, there is some evidence that the Clark Fork brown trout are the most resistant under these conditions. Evidence that supports these conclusions includes: (1) the greater sensitivity of rainbow trout than brown trout (especially Clark Fork browns) to acute metal exposure when acclimated to lower-level metals, (2) the greater sensitivity of rainbow trout when metal pulses mimic conditions in the Clark Fork River, with depressed hardness and pH, and (3) the greater sensitivity of rainbow trout in the behavioral avoidance response to metals.

Relative Lifestage Sensitivity to Clark Fork Metal Mixtures

Hatchery brown trout juveniles were slightly more tolerant than hatchery brown trout fry and substantially more tolerant than hatchery rainbow trout juveniles and fry during the pulsed exposures as demonstrated by higher survival rates. Survival rates of the different life-stages (fry and juvenile) within a species were more similar than the survival rates between species of the same lifestage (Appendix Figures 2a and 2b). We observed moderate differences in survival rates between hatchery brown trout fry and juveniles and only slight difference between hatchery rainbow trout fry and juveniles. Previous studies have shown differences in susceptibility of rainbow trout to Cu based on fish size (Howarth and Sprague 1978; Chakoumakos et al. 1979), where larger rainbow trout were more resistant to Cu. Both chinook salmon and steelhead trout showed lifestage differences in sensitivity to Zn and Cd with alevins the most resistant among the various lifestages employed (newly hatched alevins, swim-up alevins, parr, and smolts) but there were no differences among lifestages in sensitivity to Cu (Chapman 1978).

Water quality (i.e., hardness, alkalinity, pH) dramatically influenced hatchery brown and rainbow trout fry survival during pulsed exposures by generally reducing survival when hardness or hardness/pH was reduced and increasing survival when hardness was increased. Both species were most sensitive in the pulsed exposures with an initial hardness of 100 mg/L $CaCO_3$ followed by depressed hardness during the pulse (Tests II and III, Table 3). However, the influence of water hardness and/or pH did not appear uniform for the two species, as shown by comparing LC50 values and LOECs from Tests II-V to those of Test I (our base condition test) for each species (Tables 3 and 4). For instance, hatchery brown trout had either significantly higher or similar LC50 values in pulses with initial hardness 200 mg/L $CaCO_3$ followed by 100 mg/L $CaCO_3$ (Tests IV and V) compared to the base condition with constant hardness of 100 mg/L $CaCO_3$ (Test I). In contrast, hatchery rainbow trout had significantly lower LC50 values in Tests IV and V compared to Test I (Table 3). These comparisons suggest that the higher initial hardness (i.e., 200 mg/L $CaCO_3$) provides protection of hatchery brown trout from the Clark Fork metal mixture. Additionally, the moderating influence of hardness on metal toxicity may differ for the two species.

In general, most studies show that metals toxicity is reduced with increased hardness, alkalinity and pH (e.g., Alabaster and Lloyd 1980; Everall et al. 1989). Our results, along with similar findings in the published literature, are generally consistent with current theories about the effect of alkalinity, pH and hardness on metal speciation and bioavailability.

Hatchery rainbow trout fry were more sensitive in the pulsed exposures having 6 hour pulse durations than in 4 hour pulse durations. The magnitude, duration, and frequency of episodic events are all important factors determining extent of toxic response, especially in more sensitive species. For example, the effect of pH on brook trout was related to the number and magnitude of the acidic episodes (Cleveland et al. 1991). Post-pulse mortality by rainbow trout briefly exposed to 1 - 100 mg Cd/L depended upon the duration of the exposure

(Pascoe and Shazili 1986). We found rainbow trout fry were more sensitive than brown trout fry to exposure duration, providing additional evidence that rainbow trout are more sensitive than brown trout to pulse exposures of the Clark Fork metal mixture.

Delayed Mortality from Episodic Metal Exposures

In each of the pulse toxicity tests, all species and lifestages demonstrated dramatic "post-pulse" mortality following the end of the pulsed exposures. For example, hatchery rainbow trout fry survived (100% survival) throughout the 8-hour metal pulse but all died (0% survival) during the 96-hour post-pulse observation period (Test II, Appendix Figure 1b). Pascoe and Shazili (1986) also observed irreversible toxic effects initiated during pulse exposures leading to death after toxicant removal.

Hence, initial survival after a brief metal episode does not ensure continued survival, due to irreversible structural and physiological injury that can lead to death. For example, sticklebacks exposed for 16-hours to approximately 1.0 mg/L Zn demonstrated extensive gill damage even after five days in control water (Matthiessen and Brafield 1973). Disruption or alteration of gill function (i.e., chloride cell function) causes ionic and osmotic imbalances that are particularly stressful to freshwater fishes (Eddy 1981, 1982).

Exposure to metal pulses may also cause behavioral impairments that reduce locomotor and feeding behavior; Cleveland et al. (1991) observed reduced swimming and feeding behaviors (i.e., prey encounters, prey-capture efficiency, and predator avoidance) by brook trout exposed to a pulse of aluminum. Early lifestages that are highly dependent upon rapid growth and development are particularly sensitive to slight variations in feeding and swimming efficiency (Cleveland et al. 1991). The reduced numbers of early lifestages of trout in the Clark Fork River could in part, be explained by behavioral impairments that follow episodic exposure to metals.

In summary, pulsed metal exposures representing episodic events observed in the Clark Fork River are lethal to both early and juvenile lifestages of brown and rainbow trout.

Delayed mortality following the pulse events in the laboratory suggests that these episodic metal exposures can significantly reduce survival in the wild and thus, lower recruitment of sub-adults into the resident trout populations.

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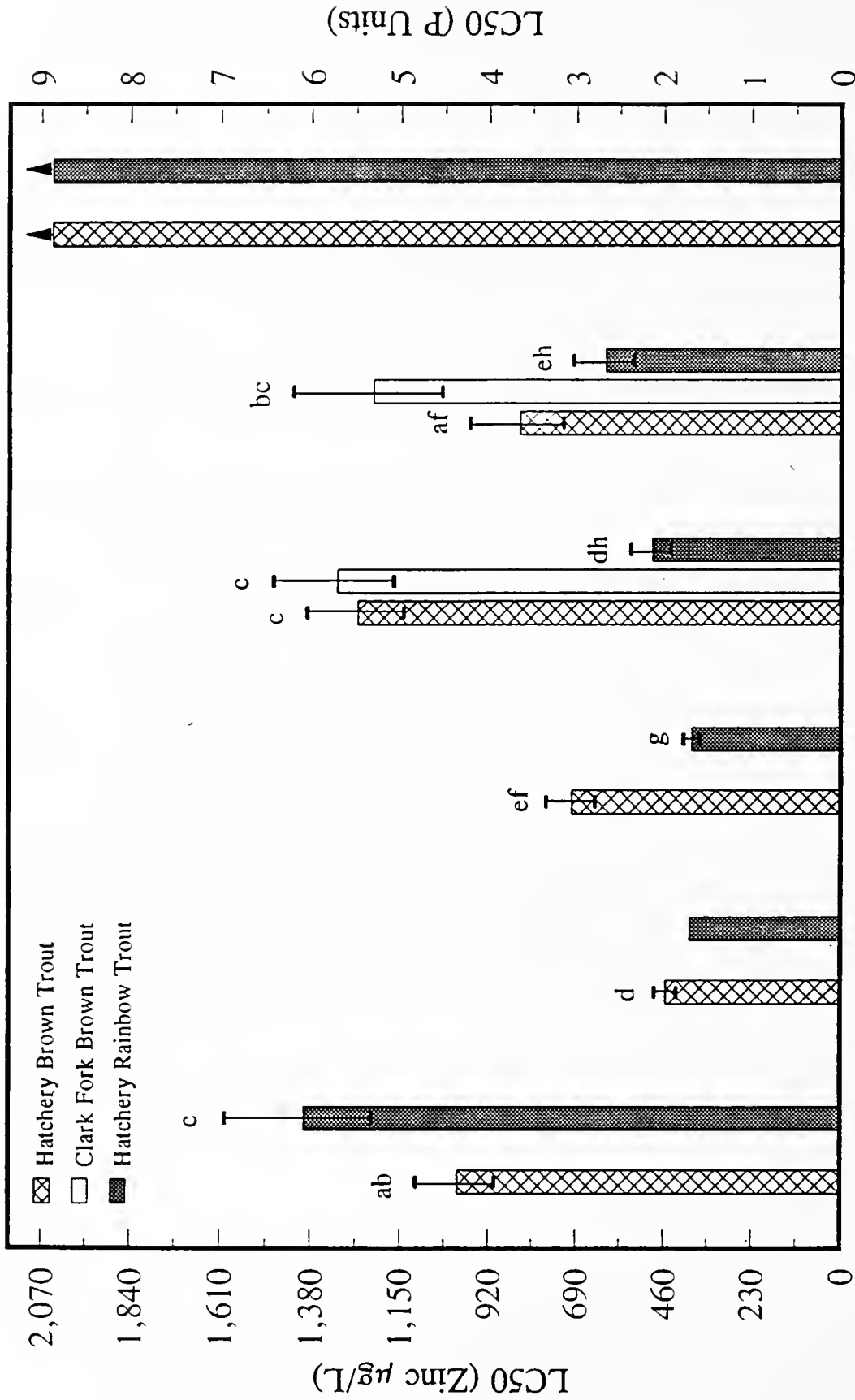Zar, J.H. 1984. Biostastical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 718 p.

Figure 1. Median lethal concentrations (LC50) and 95% confidence intervals (µg Zn/L and P units) for hatchery brown trout fry, hatchery rainbow trout fry, and Clark Fork brown trout fry from the eight hour (1,6,1-hour; see text) pulsed exposures to dilutions of a mixture of Zn, Cu, Pb, and Cd. LC50 values based on the measured Zn concentrations (µg/L) and converted into P Units, where $1P = 230$ Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Nominal hardness (ppm CaCO$_3$) and measured average pH are shown for each test (Tests I - VI) as determined prior to/during peak metals exposure (Also see Appendix Table 1). For comparisons, LC50 values (µg Zn/L) shown with the same letter superscript are not significantly different, based on comparison of 95% confidence limits ($\alpha = 0.05$) with overall protection of $(0.95)^n$, where n=the number of species/strains being compared. Also see Table 3.

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Table 1. Metal concentrations measured in the upper Clark Fork River (CFR), Montana, during storm "pulse" events and "redwater" spill events (concentrations in µg/L (ppb) total recoverable unless otherwise noted).

Date	Sample collection location	Event	Metal concentration (µg/L)			
			Zn	Cu	Pb	Cd
March 10, 1960[a]	CFR below Warm Spring Ponds	redwater	—	9,000	—	—
	CFR above East Missoula	redwater	—	3,100	—	—
May 1, 1968	CFR between Garrison and Deer Lodge	redwater	4.3	620	—	—
Nov. 20, 1968	CFR near Warm Springs	redwater	32,500	4,000	—	—
April 10, 1969	CFR at Deer Lodge	redwater	3,600	1,100	—	—
March 1, 1972	CFR near Warm Springs	redwater	500	820	—	—
May 27, 1988[b]	Mill-Willow Bypass	storm event	—	2,480	—	—
July 12, 1989[b]	Mill-Willow Bypass	storm event	14,000	13,300	30	85
	CFR below Warm Springs		800	450	10	6
	CFR at Perkins Lane		120	180	4	3
	CFR near Galen		210	370	2	3
	CFR at Deer Lodge (total recoverable)		560	330	15	3
	(dissolved)		230	120	<1	2
July 2, 1990[b]	Mill-Willow Bypass	storm event	10,300	5,800	—	—

[a] Water samples collected in association with mortality to caged fish placed instream; no dead native fish observed.
[b] Water samples collected and analyzed in association with a documented fish kill.

Table 2. Nominal hardness, alkalinity and pH conditions, prior to, following (Pre/Post), and during the pulse (Pulse) of metals for Tests I - VI; and fry species/strains used in each test (hatchery brown trout, H-BNT, hatchery rainbow trout, H-RBT, or Clark Fork brown trout, CF-BNT). Changes in hardness and pH during a test are symbolized as: c = constant, ↓ = depressed, ↓↓ = highly depressed, and ↑↑ = highly elevated.

Test	Hardness (ppm $CaCO_3$)		Alkalinity (ppm $CaCO_3$)		pH		Species/Strain
	Pre/Post	Pulse	Pre/Post	Pulse	Pre/Post	Pulse	
I c Hardness c pH	100	100	100	100	7.2-8.0	7.2-8.0	H-BNT, H-RBT
II ↓ Hardness c pH	100	50	100	50	7.2-8.0	7.2-8.0	H-BNT, H-RBT
III ↓ Hardness ↓ pH	100	50	100	0	7.2-8.0	4.5	H-BNT, H-RBT
IV ↓↓ Hardness ↓ pH	200	100	180	0	7.2-8.0	4.5	H-BNT, H-RBT, CF-BNT
V ↓ Hardness ↓ pH	200	100	180	0	7.2-8.0	4.5	H-BNT, H-RBT, CF-BNT
VI ↑↑ Hardness ↓ pH	200	400	180	0	7.2-8.0	4.5	H-BNT, H-RBT

Table 3. LC50 estimates (95% confidence limits in parentheses, μg Zn/L) for hatchery brown trout fry, hatchery rainbow trout fry, and Clark Fork brown trout fry from the eight hour (1,6,1 -hour; see text) pulsed exposures to dilutions of a mixture of Zn, Cu, Pb, and Cd. Average hardness (ppm CaCO$_3$), alkalinity (ppm CaCO$_3$), and pH are shown for each test (Tests I - VI) as measured during peak metals exposure (Also see Appendix Table 1). LC50 values based on the measured Zn concentrations (μg/l) in the 1P metals mixture, where 1P nominal concentrations (μg/L) for the metals are as follows: 230 Zn, 120 Cu; 3.2 Pb, and 2.0 Cd. LC50 calculations made using the Spearman-Karber method (Hamilton et al. 1977). For comparisons, LC50 values (μg Zn/L) shown with the same letter superscript are not significantly different, based on comparison of 95% confidence limits ($\alpha=0.05$) with overall protection of $(0.95)^n$, where n= the number of species/strains being compared. Also see Figure 1.

Test	Average Mid-Pulse Conditions			Zinc LC50 (95% confidence limits)		
	Hardness (ppm CaCO$_3$)	Alkalinity (ppm CaCO$_3$)	pH	Hatchery brown trout	Hatchery rainbow trout	Clark Fork brown trout
I	108.8	105.0	7.58	1,000 (903 - 1,108) [ab]	1,397 (1,222 - 1,597) [c]	
II	64.5	50.3	7.30	454 (426 - 484) [d]	391 (no c.l.)	
III	47.1	1.4	4.84	700 (639 - 767) [ef]	386 (367 - 407) [g]	
IV	119.4	2.3	5.12	1,260 (1,142 - 1,390) [c]	490 (440 - 546) [dh]	1,312 (1,167 - 1,475) [c]
V	112.5	5.4	5.24	838 (724 - 970) [af]	614 (540 - 698) [eh]	1,220 (1,043 - 1,426) [bc]
VI	423.9	4.2	4.96	>2,042 (no c.l.)	>2,042 (no c.l.)	

Table 4. The highest concentration of metals (P units, see text) having no statistically significant adverse effect on survival (NOEC, no observed effect concentration) and the lowest concentration of metals having a statistically significant adverse effect on survival (LOEC, lowest observed effect concentration) of hatchery brown trout fry, hatchery rainbow trout fry, and Clark Fork brown trout fry following the eight hour pulsed exposures (Tests I - VI). NOECs and LOECs were determined by Dunnett's comparisons of treatment survival means to control survival means. The nominal 1P concentrations (μg/L) are as follows: 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. nP refers to the dilution of the above metals concentration. Changes in hardness and pH during each test are symbolized as: ¢ = constant, ↓ = depressed, ↓↓ = highly depressed, and ↑↑ = highly elevated. Also see Table 2 and Appendix Table 6.

Test	Hatchery brown trout		Hatchery rainbow trout		Clark Fork brown trout	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
I ¢ Hardness ¢ pH	2P	4P	2P	4P		
II ↓ Hardness ¢ pH	1P	2P	<1P	1P		
III ↓ Hardness ↓ pH	1P	2P	<1P	1P		
IV ↓↓ Hardness ↓ pH	2P	4P	<1P	1P	2P	4P
V ↓↓ Hardness ↓ pH	2P	4P	1P	2P	4P	8P
VI ↑↑ Hardness ↓ pH	8P	>8P	4P	8P		

Table 5. LC50 estimates (95% confidence limits in parentheses, μg Zn/L) for fry and juvenile hatchery brown and rainbow trout from the eight hour (2,4,2 - hour; see text) pulsed exposures to dilutions of a mixture of Zn, Cu, Pb, and Cd. Average hardness (ppm $CaCO_3$), alkalinity (ppm $CaCO_3$), and pH were 122.5, 2.6, and 4.9, respectively, for combined tests (Tests VII and VIII) as measured during peak metals exposure. LC50 values based on the measured Zn concentrations (μg/L) in the 1P metals mixture, where 1P nominal concentrations (μg/L) for the metals are as follows: 230 Zn, 120 Cu; 3.2 Pb, and 2.0 Cd. LC50 calculations made using the Spearman-Karber method (Hamilton et al. 1977). For comparisons, LC50 values (μg Zn/L) shown with the same letter superscript are not significantly different, based on comparison of 95% confidence limits ($\alpha = 0.05$) with overall protection of $(0.95)^4 = 0.81$.

Species/Age	Zinc LC50 (95% confidence limits)
Hatchery brown trout/Fry	1,041 (816 - 1,329) [a]
Hatchery brown trout/Juvenile	> 1,856 (no c.l.)
Hatchery rainbow trout/Fry	738 (643 - 848) [ab]
Hatchery rainbow trout/Juvenile	691 (646 - 739) [b]

Table 6. The highest concentration of metals (P units, see text) having no statistically significant adverse effect on survival (NOEC, no observed effect concentration) and the lowest concentration of metals having a statistically significant adverse effect on survival (LOEC, lowest observed effect concentration) of hatchery brown trout fry, hatchery brown trout juveniles, hatchery rainbow trout fry, and hatchery rainbow trout juveniles following the eight hour pulsed exposures (Tests VII and VIII combined, see text). NOECs and LOECs were determined by Dunnett's comparisons of treatment survival means to control survival means. The nominal 1P concentrations (μg/L) are as follows: 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. nP refers to the dilution of the above metals concentration. Also see Appendix Table 7.
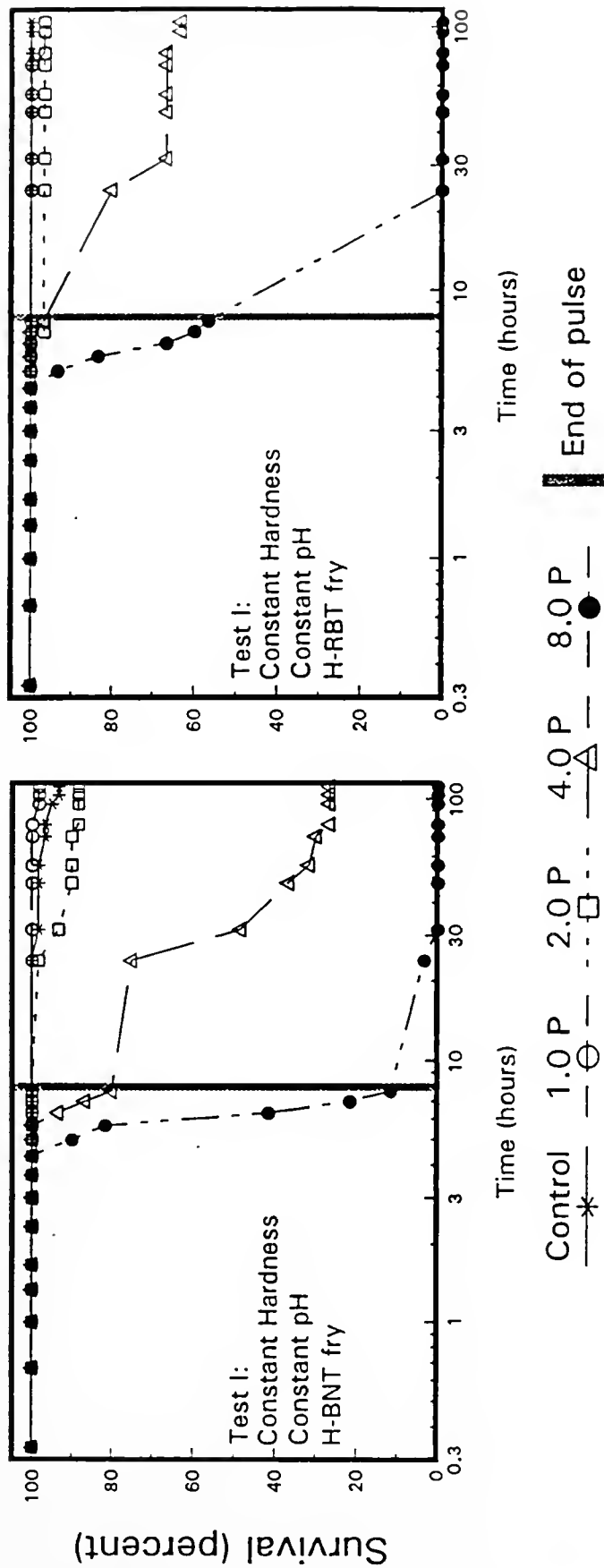
Species/Age	NOEC	LOEC
Hatchery brown trout/Fry	2P	4P
Hatchery brown trout/Juvenile	4P	8P
Hatchery rainbow trout/Fry	2P	4P
Hatchery rainbow trout/Juvenile	2P	4P

Table 7. Relative sensitivities of hatchery brown and rainbow trout as determined by the measured responses used in the various exposure conditions from the Fishery Protocols #1,2,3,4 and 5, specified under Research Plans, Section 7.4.4, in the Assessment Plan: Part I, Clark Fork River NPL Sites, Montana (Montana 1992).

Fishery Protocol	Exposure conditions	Response Endpoint	Observed Response	Relative species/ strain sensitivity
5	Acutely lethal concentrations of Clark Fork metals mixture (Range = 0 to 5P, where 1P = 230 Zn, 120 Cu, 3.2 Pb and 2.0 Cd)	96-hr LC50	Death	CF-BNT > H-BNT > H-RBT
5	same	12-hr LC50	"	CF-BNT = H-BNT = H-RBT
5	Acutely lethal concentrations of Clark Fork metals mixture (1P challenge concentration) after acclimation to 0.0P (control) metals	Mean time to death - after 3 weeks accl. - after 5 weeks accl.	" "	H-BNT > H-RBT = CF-BNT H-BNT = H-RBT > CF-BNT
5	Acutely lethal concentrations of Clark Fork metals mixture (1P challenge concentration) after acclimation to 0.2P metals	Mean time to death - after 3 weeks accl. - after 5 weeks accl. - after 2 weeks de-accl.	" " "	H-BNT = H-RBT > CF-BNT H-RBT > H-BNT > CF-BNT H-BNT > H-RBT
3	Acutely lethal pulse concentrations of Clark Fork metal mixture (Range = 0 to 8P) for 8 hr pulse + 96 hr post-pulse at 0.0P Pulsed exposures using fry only: - Constant hardness (100 mg/L) and constant pH (7.58) during pulse	8 + 96-hr LC50	"	H-BNT > H-RBT
	- Reduced hardness (100 to 50 mg/L) and constant pH (7.30) during pulse	8 + 96-hr LC50	"	H-RBT ≥ H-BNT
	- Reduced hardness (100 to 50 mg/L) and reduced pH (7.71 to 4.84) during pulse	8 + 96-hr LC50	"	H-RBT >> H-BNT

Table 7 (continued).

Fishery Protocol	Exposure conditions	Response Endpoint	Observed Response	Relative species/ strain sensitivity
3	- Reduced hardness (200 to 100 mg/L) and reduced pH (7.90 to 5.12) during pulse	8 + 96-hr LC50	Death	H-RBT >> H-BNT = CF-BNT
	- Reduced hardness (200 to 100 mg/L) and reduced pH (7.87 to 5.24) during pulse	8 + 96-hr LC50	"	H-RBT > H-BNT > CF-BNT
	- Elevated hardness (200 to 400 mg/L) and reduced pH (7.86 to 4.96) during pulse	8 + 96-hr LC50	"	H-BNT = H-RBT
	Pulsed exposures using juveniles (j) and fry (f): - Reduced hardness (200 to 100 mg/L) and reduced pH (7.87 to 4.83/5.05) during pulse	8 + 96-hr LC50	"	H-RBT j ≥ H-RBT f ≥ H-BNT f > H-BNT j
1	Chronic 88-day food/water exposure to Warm Springs diet vs. Turah Bridge diet	88-day growth	Reduced growth	H-RBT ≥ H-BNT
1,2	same	Physiology	Gut impaction	H-BNT > H-RBT
1,2	same	Physiology	Lipid peroxidation	H-BNT > H-RBT
1,2	same	Physiology	Histopathology	H-BNT > H-RBT
4	Short-term exposure to paired control and metal contaminated water in avoidance chamber (Range of metal mixture = 0.1 to 10 X)	Behavior	Avoidance	H-RBT >> H-BNT

Appendix Figure 1a - 1f. Cumulative percent survival throughout the 8-hour pulse and 96-hour post-pulse observation periods for hatchery brown trout fry (H-BNT), hatchery rainbow trout fry (H-RBT) and Clark Fork brown trout fry (CF-BNT) exposed to dilutions of a mixture of Zn, Cu, Pb and Cd. Exposure dilutions contained either 0P-control, 1P, 2P, 4P or 8P metal concentrations where the nominal metal concentrations (μg/L) in the 1P mixture = 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. P Units represent the notation for observed metal concentrations during pulse events in the Clark Fork River (see text). Water quality conditions (hardness, pH) for each test (Tests I - VI) were as described in Appendix Table 1.
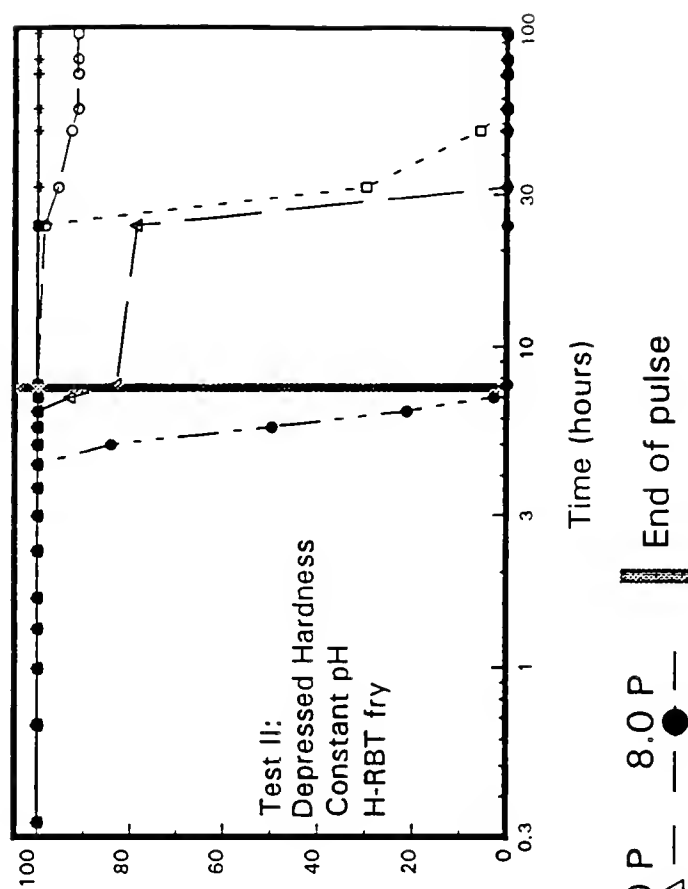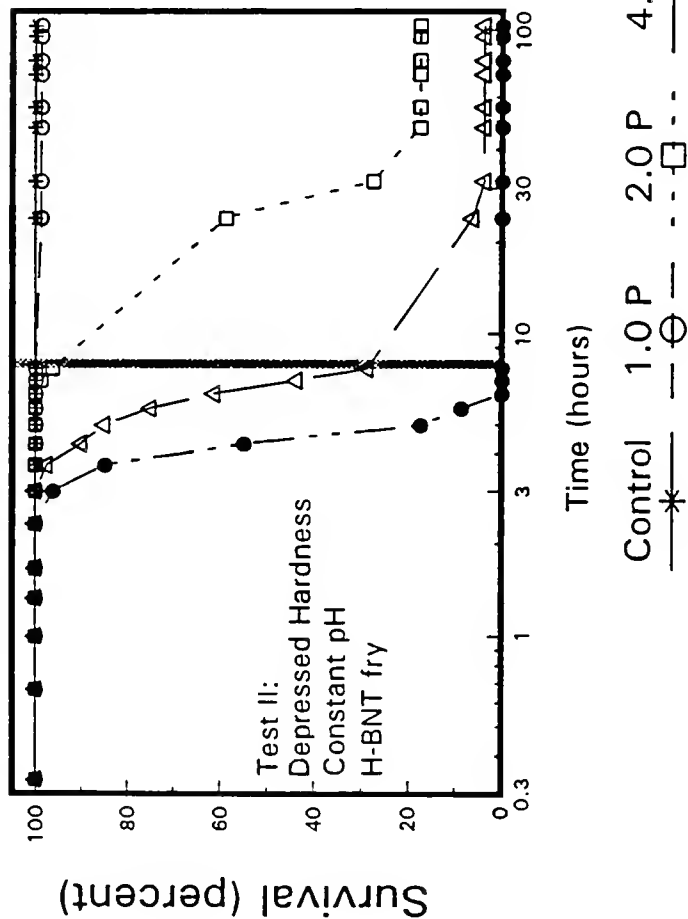
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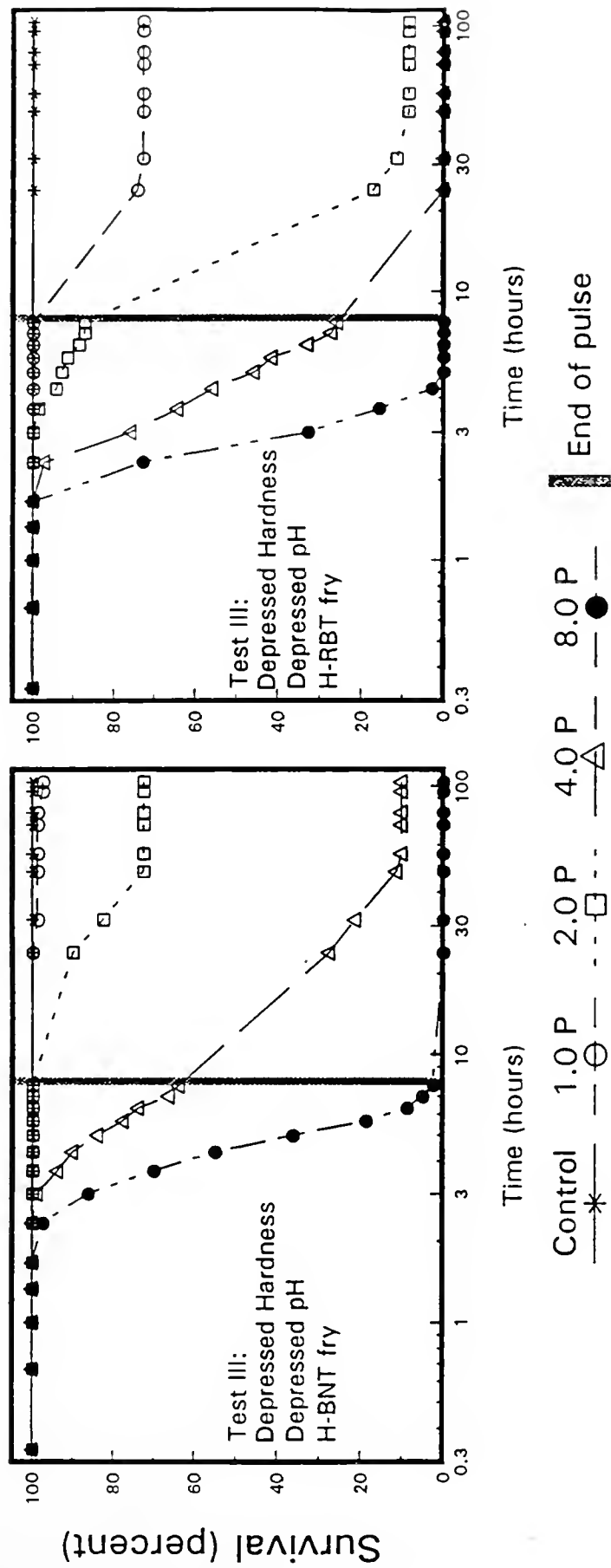
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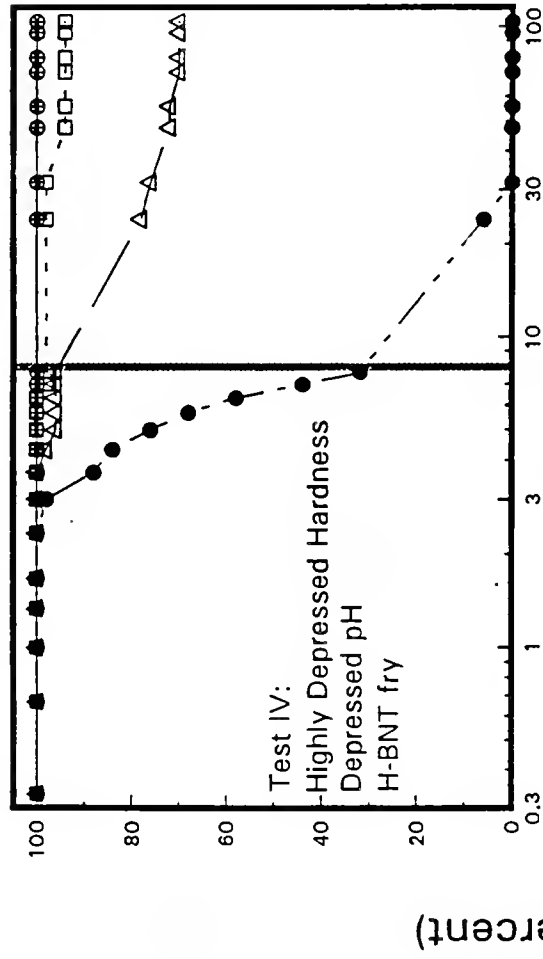
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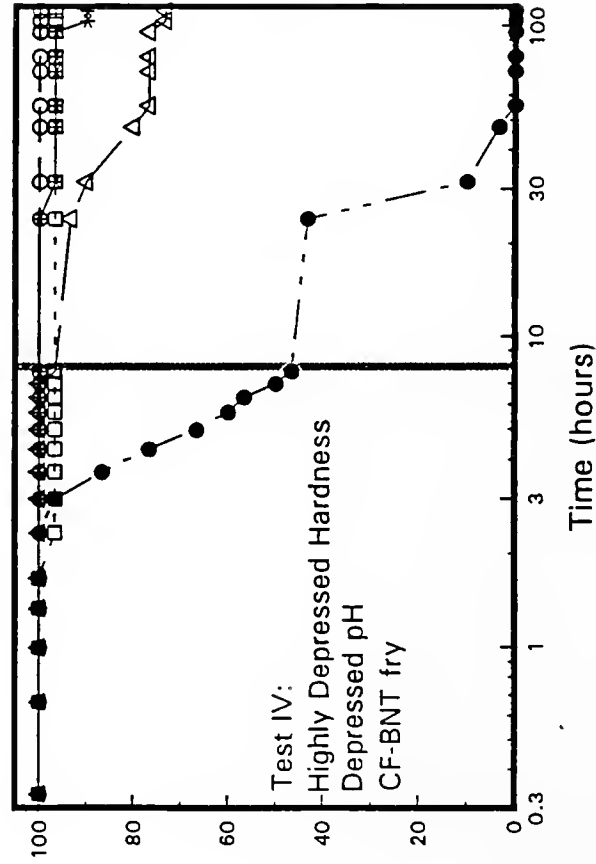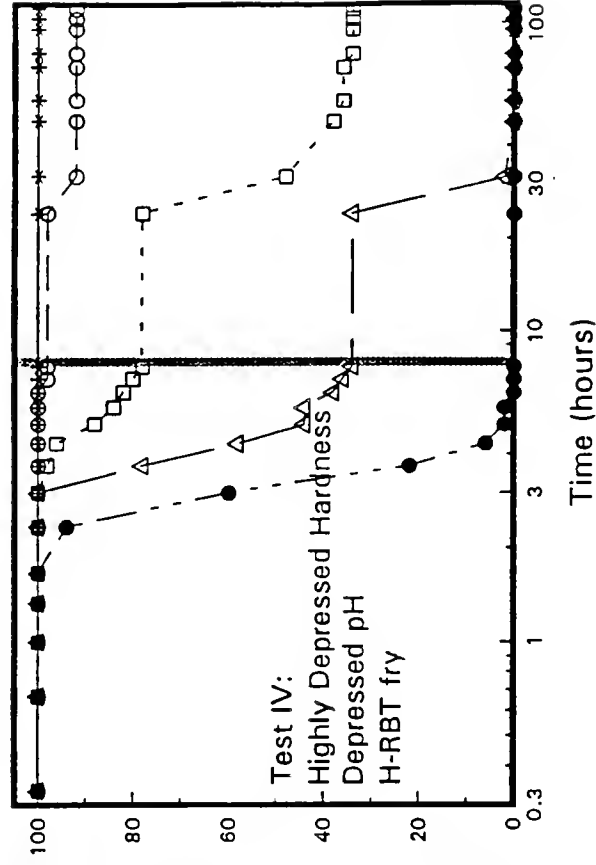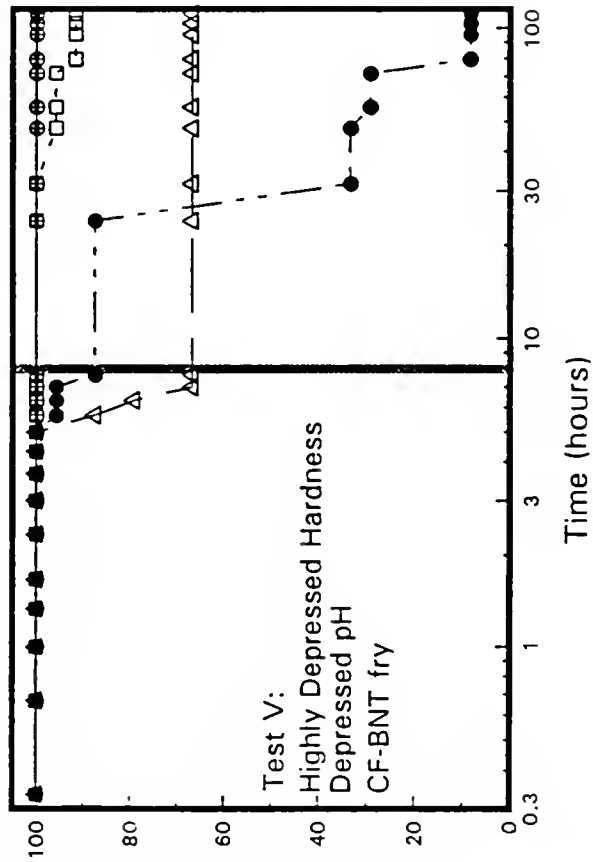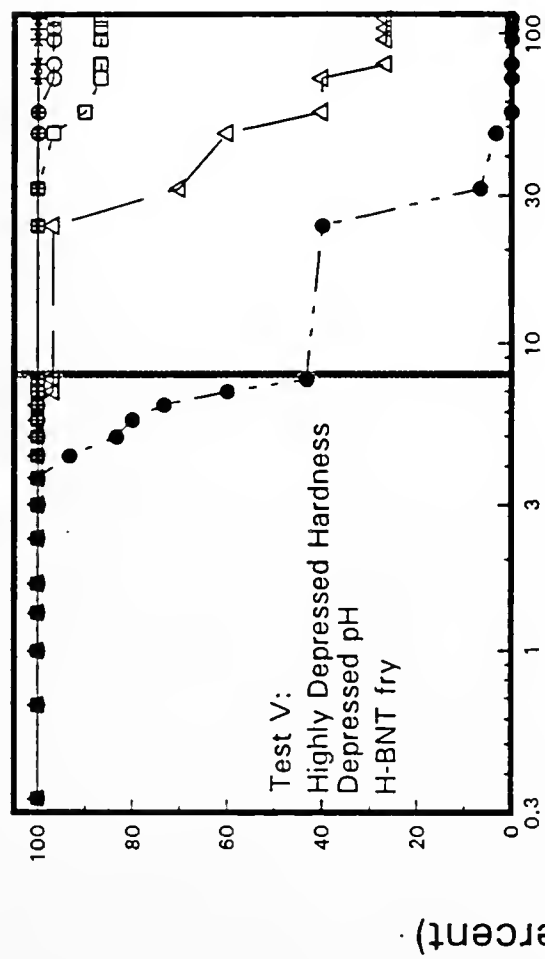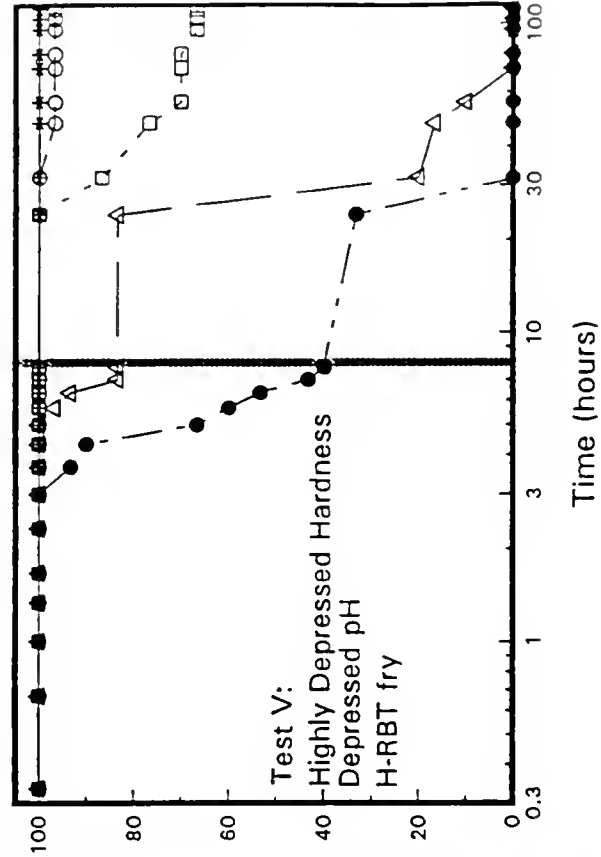
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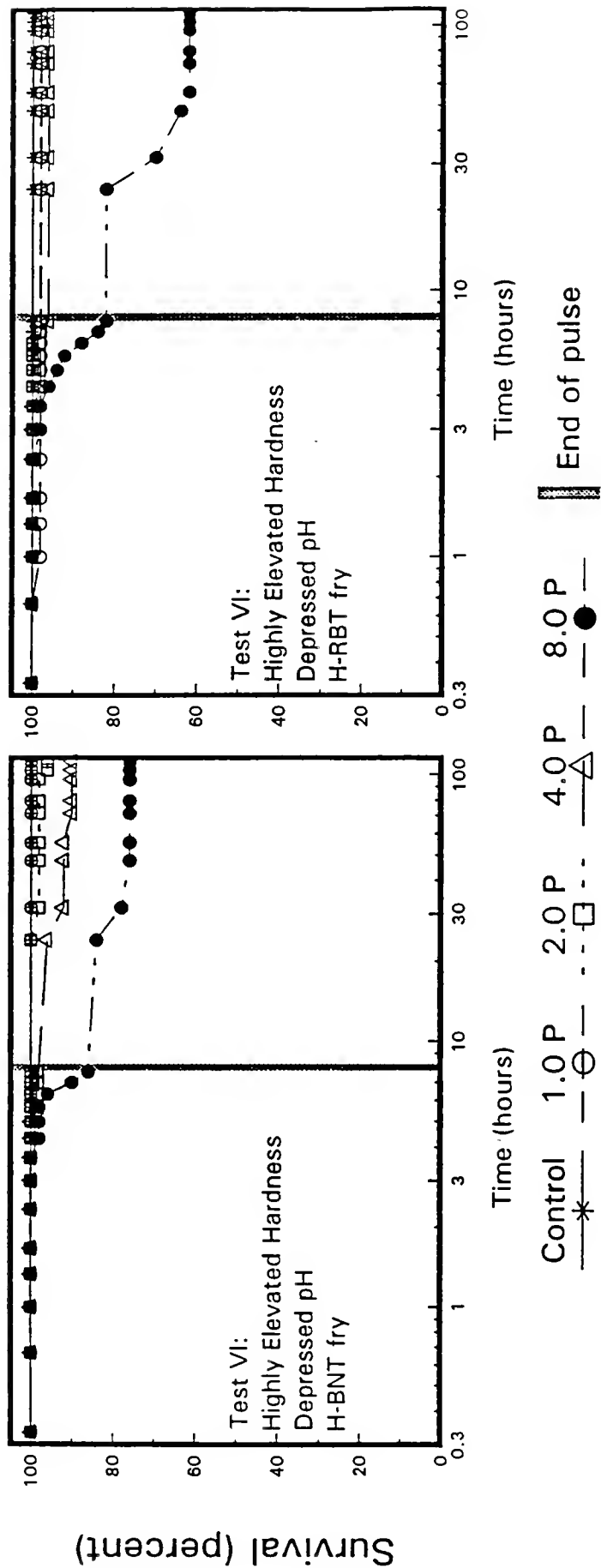
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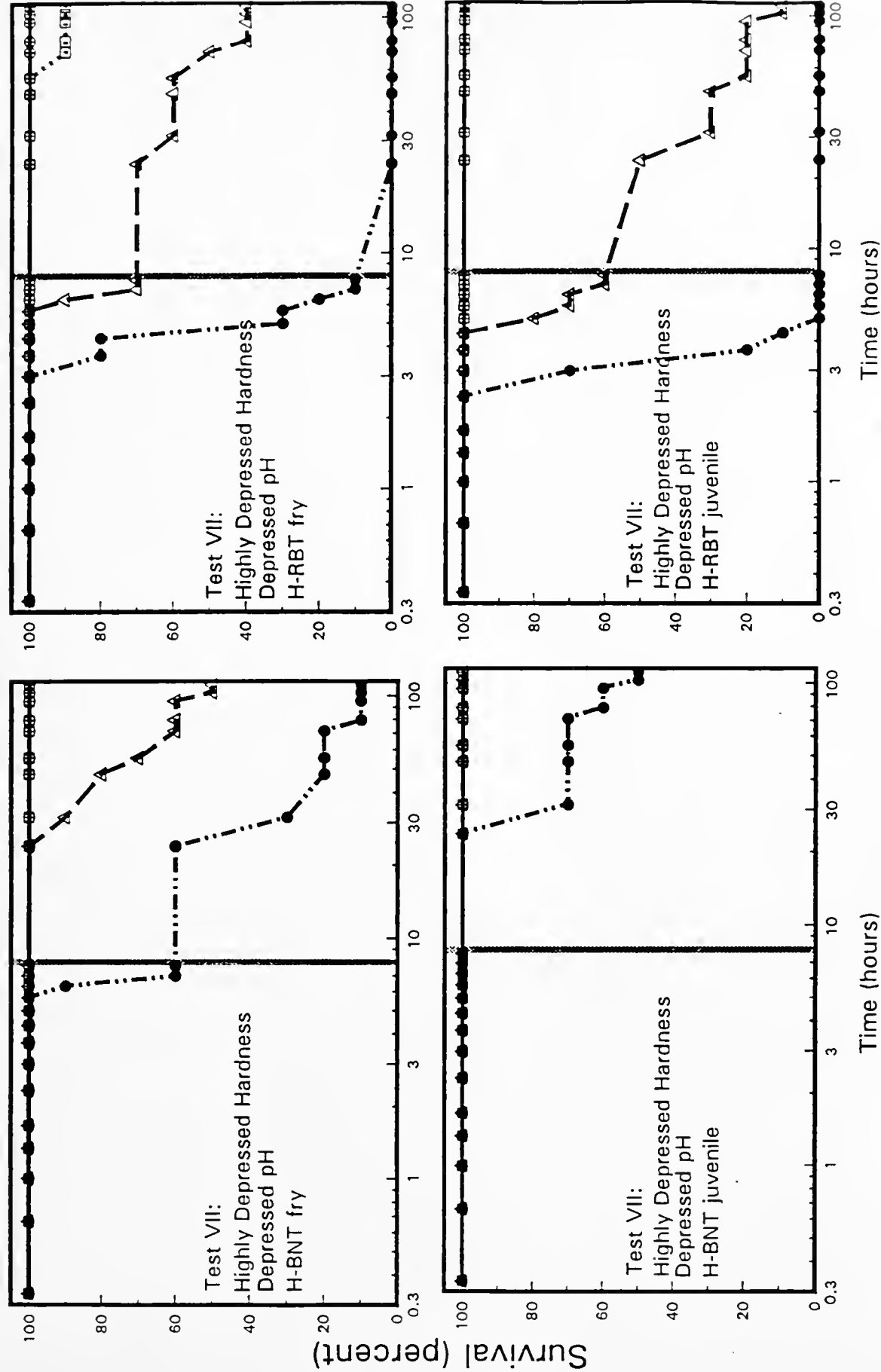
Appendix Figure 2a and 2b. Cumulative percent survival throughout the 8-hour pulse and 96-hour post-pulse observation periods for the different lifestages of hatchery brown trout (H-BNT fry and H-BNT juvenile) and hatchery rainbow trout (H-RBT fry and H-RBT juvenile) exposed to dilutions of a mixture of Zn, Cu, Pb and Cd. Exposure dilutions contained either 0P-control, 1P, 2P, 4P or 8P metal concentrations where the nominal metal concentrations (µg/L) in the 1P mixture = 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. P Units represent the notation for observed metal concentrations during pulse events in the Clark Fork River (see text). Water quality conditions (hardness, pH) for each test (Tests VII and VIII) were as described in Appendix Table 1.
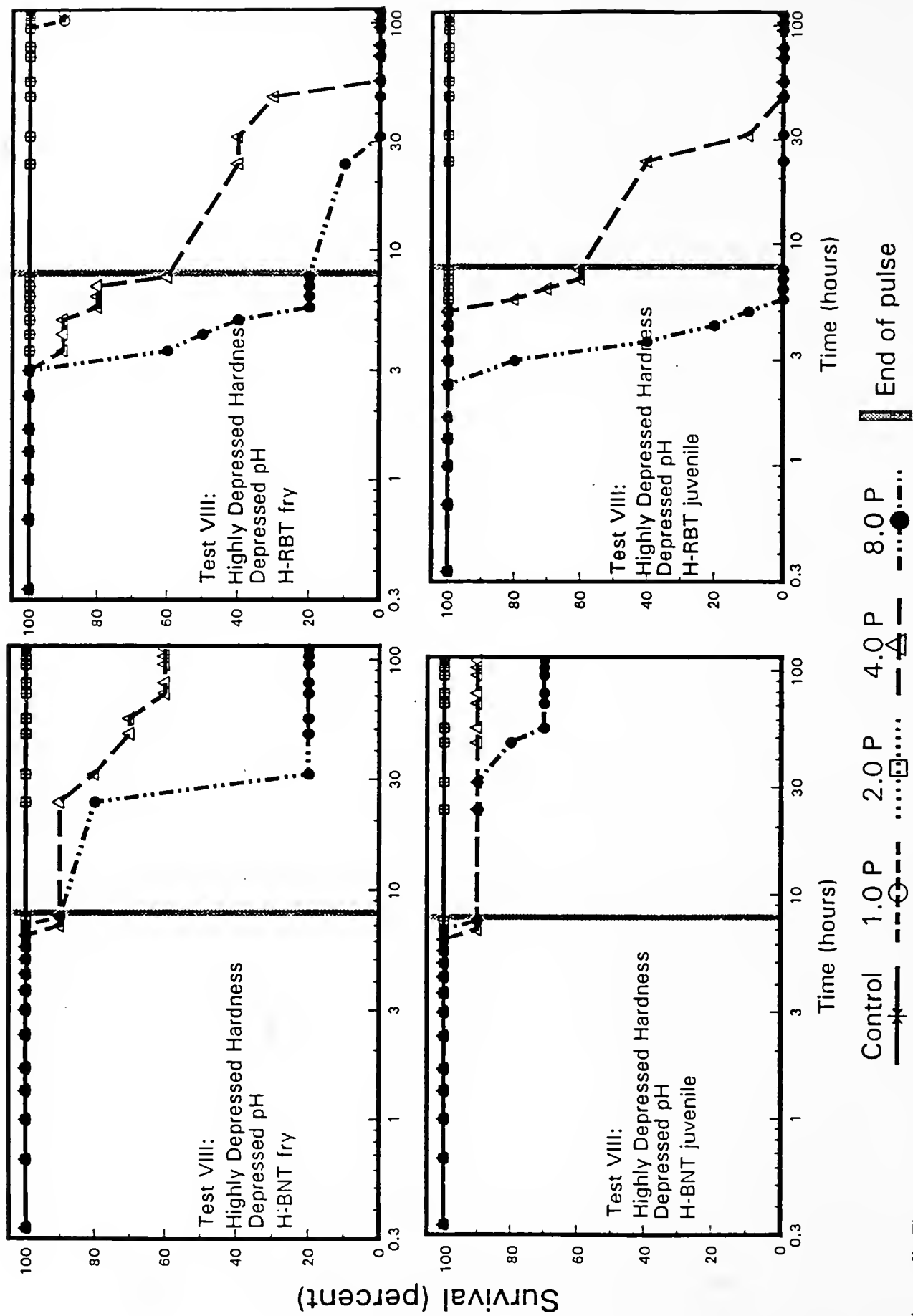
Appendix Figure 2b (continued).

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Appendix Table 1. Water quality conditions from the pulse experiments expressed for nominal level, measured average, respective range and sample size (N) for hardness (ppm $CaCO_3$), alkalinity (ppm $CaCO_3$), and pH.

		Pre/Post-Pulse Conditions				Mid-Pulse Conditions			
		Nominal	Avg.	Range	N	Nominal	Avg.	Range	N
TEST I	Hard.	100	104.4	(100.0 - 112.0)	11	100	108.8	(104.0 - 112.0)	5
	Alk.	80 - 110	98.5	(92.0 - 104.0)	8	80 - 110	105.0	(102.0 -108.0)	5
	pH	7.2 - 8.0	7.45	(7.30 - 7.60)	8	7.2 - 8.0	7.58	(7.40 - 7.70)	5
TEST II	Hard.	100	105.9	(96.0 -120.0)	30	50	64.5	(44.0 - 122.0)	27
	Alk.	80 - 110	102.2	(94.0 - 120.0)	24	40 - 60	50.3	(46.0 - 56.0)	6
	pH	7.2 - 8.0	7.61	(7.30 - 7.80)	30	7.2 - 8.0	7.30	(7.20 - 7.40)	6
TEST III	Hard.	100	102.2	(93.4 - 109.6)	30	50	47.1	(40.6 - 55.0)	24
	Alk.	80 - 110	94.5	(90.0 - 102.0)	15	0 - 10	1.4	(0.9 - 2.0)	4
	pH	7.2 - 8.0	7.71	(7.52 - 7.85)	30	4.5	4.84	(4.45 - 5.90)	25
TEST IV	Hard.	200	203.4	(190.8 - 212.0)	30	100	119.4	(108.0 - 176.0)	25
	Alk.	180 - 200	186.5	(182.0 - 192.0)	15	0 - 10	2.3	(0.4 - 8.0)	14
	pH	7.2 - 8.0	7.90	(7.68 - 8.09)	30	4.5	5.12	(4.38 - 6.38)	25
TEST V	Hard.	200	202.3	(194.5 - 209.1)	28	100	112.5	(98.2 - 127.9)	25
	Alk.	180 - 200	184.4	(178.0 - 190.0)	18	0 - 10	5.4	(1.0 - 17.0)	10
	pH	7.2 - 8.0	7.87	(7.55 - 8.09)	27	4.5	5.24	(4.63 - 6.19)	25

Appendix Table 1 (continued).

| | | Pre/Post-Pulse Conditions | | | | Mid-Pulse Conditions | | |
	Nominal	Avg.	Range	N	Nominal	Avg.	Range	N
TEST VI								
Hard.	200	207.2	(196.3 - 220.3)	30	400	423.9	(403.7 - 452.9)	25
Alk.	180 - 200	185.6	(176.0 - 192.0)	15	0 - 10	4.2	(0.9 - 10.0)	10
pH	7.2 - 8.0	7.86	(7.62 - 8.10)	30	4.5	4.96	(4.61 - 5..65)	25
TEST VII								
Hard.	200	204.5	(195.8 - 216.2)	25	100	110.6	(106.1 - 118.3)	20
Alk.	180 - 200	185.5	(177.4 - 192.0)	13	0 - 10	2.2	(0.97 - 5.85)	8
pH	7.2 - 8.0	7.87	(7.66 - 8.20)	25	4.5	4.83	(4.25 - 5.46)	20
TEST VIII								
Hard.	200	207.3	(199.7 - 220.5)	30	100	134.4	(108.2 - 199.7)	19
Alk.	180 - 200	185.3	(179.4 - 191.1)	18	0 - 10	2.9	(1.95 - 3.98)	6
pH	7.2 - 7.8	7.86	(7.69 - 8.11)	30	4.5	5.05	(4.48 - 5.58)	20

Appendix Table 2. Average dissolved oxygen (ppm and % saturation) and temperature (°C) with respective ranges and sample sizes (N) from the pulse experiments; values represent measurements taken frequently during the actual pulse and then once daily during 96 hour post-pulse period.

	Oxygen				Temperature		
	(ppm)	(range)	(%)	(N)	(°C)	(range)	(N)
TEST I	6.8	(6.4 - 7.2)	79	16	10.2	(10.0 - 10.5)	16
TEST II	7.1	(6.7 - 7.4)	84	40	10.3	(10.0 - 11.0)	40
TEST III	7.0	(6.8 - 7.3)	83	40	10.3	(10.0 - 11.0)	40
TEST IV	7.0	(6.5 - 7.6)	83	45	10.2	(9.8 - 11.5)	45
TEST V	7.0	(6.8 - 7.3)	83	40	10.2	(10.0 - 10.8)	40
TEST VI	6.9	(6.5 - 7.2)	81	45	10.2	(9.8 - 10.8)	45
TEST VII	8.1	(7.8 - 8.3)	94	40	10.1	(10.0 - 10.5)	40
TEST VIII	8.1	(7.8 - 8.3)	94	40	10.0	(9.7 - 10.5)	40

Appendix Table 3. Measured Zn (μg/L) concentrations in test (8P, 4P, 2P and 1P) and control (C) waters prior to (Pre-Pulse), during (Mid-Pulse) and following (Post-Pulse) the pulsed exposures (Tests I - VIII). P Units represent the notation for observed metal concentrations in the Clark Fork River (see text), where metal concentrations (μg/L) for 1P are 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Average Zn (μg/L) concentrations (standard deviation, S.D.; and sample size, N = 2 unless otherwise indicated) determined by atomic absorption spectroscopy. Values below the method detection limit (in μg/L) of 4.9 are represented as "<.".

	Treatment (P Units)	Pre-Pulse Mean (S.D.)	Mid-Pulse Mean (S.D.)	Post-Pulse Mean (S.D.)
TEST I	8	<. (__)	2537.6 (48.7; 10)	25.7 (7.8; 8)
	4	5.8 (4.5)	1294.9 (68.6; 8)	27.5 (8.0; 8)
	2	8.2 (2.1)	628.1 (35.0; 9)	24.3 (8.2; 8)
	1	<. (__)	321.6 (21.8; 9)	24.0 (7.2; 8)
	C	5.2 (1.7)	22.2 (15.8; 9)	23.3 (9.1; 8)
TEST II	8	65.1 (3.0)	2294.5 (149.1; 16)	6.1 (17.3; 8)
	4	61.6 (4.6)	1216.3 (39.4; 15)	8.7 (21.57; 10)
	2	56.4 (6.9)	577.0 (24.2; 16)	16.5 (20.1; 10)
	1	59.7 (3.7)	283.2 (17.8; 16)	<. (__; 10)
	C	63.3 (7.7)	23.6 (15.0; 15)	10.8 (18.1; 10)
TEST III	8	17.8 (0.4)	2290.8 (178.3; 16)	<. (__; 10)
	4	30.8 (23.0)	1151.0 (89.8; 16)	<. (__; 10)
	2	19.5 (7.1)	566.0 (20.6; 16)	5.8 (5.3; 10)
	1	55.8 (25.8)	270.7 (20.6; 16)	13.1 (14.6; 10)
	C	33.8 (10.3)	14.3 (13.3; 16)	8.8 (17.0; 10)
TEST IV	8	9.8 (1.8)	2170.3 (179.4; 15)	6.2 (4.9: 10)
	4	14.5 (3.5)	1186.3 (120.2; 16)	5.9 (6.1; i0)
	2	9.5 (2.1)	569.4 (8.8; 16)	6.1 (3.9; 10)
	1	12.3 (11.0)	300.4 (10.5; 16)	6.3 (6.3; 10)
	C	8.8 (1.1)	8.6 (7.5; 16)	5.2 (4.3; 10)
TEST V	8	<. (__)	2024.5 (123.5; 16)	<. (__; 10)
	4	<. (__)	1086.0 (50.2; 16)	<. (__; 10)
	2	<. (__)	551.7 (12.8; 16)	<. (__; 10)
	1	<. (__)	276.4 (9.0; 16)	<. (__; 10)
	C	<. (__)	<. (__; 16)	<. (__; 10)
TEST VI	8	<. (__)	2042.3 (251.1; 8)	<. (__; 10)
	4	6.0 (0.5)	1040.4 (50.2; 7)	<. (__; 10)
	2	5.5 (1.2)	545.7 (8.2; 16)	<. (__; 10)
	1	5.7 (0.0)	302.6 (27.4; 16)	<. (__; 10)
	C	<. (__)	<. (__; 16)	<. (__; 10)

	Treatment (P Units)	Pre-Pulse Mean (S.D.)	Mid-Pulse Mean (S.D.)	Post-Pulse Mean (S.D.)
TEST VII	8	<. (__)	1814.0 (212.7; 16)	13.4 (12.9; 8)
	4	<. (__)	947.4 (31.8; 16)	19.4 (44.3; 8)
	2	6.3 (4.7)	468.1 (42.9; 16)	33.2 (44.2; 8)
	1	21.3 (__)*	251.1 (47.3; 16)	7.1 (8.0; 8)
	C	15.3 (22.2)	12.7 (20.6; 16)	6.1 (9.9; 8)
TEST VIII	8	6.0 (5.7)	1898.8 (75.3; 16)	<. (__; 10)
	4	18.8 (25.2)	958.3 (21.8; 16)	<. (__; 10)
	2	32.8 (45.5)	466.4 (47.7; 16)	<. (__; 10)
	1	<. (__)	231.2 (5.0; 16)	<. (__; 10)
	C	<. (__)	<. (__; 16)	<. (__; 10)

* indicates sample size = 1.

Appendix Table 4. Measured metal concentrations in test (8P, 4P, 2P and 1P) and control (C) waters during the pulsed exposures (Tests 1 - VIII). P Units represent the notation for observed metal concentrations in the Clark Fork River (see text), where metal concentrations (μg/L) for 1P are 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Average Cu, Pb and Cd (μg/L) maximum concentrations (standard deviation in parentheses) determined by atomic absorption spectroscopy. Values below the method detection limit (in μg/L) of 4.6 Cu, 1.7 Pb and 0.4 Cd are represented as "<."; sample size was 2 unless otherwise indicated.

	Treatment	Average Maximum Concentration		
	(P Units)	Cu	Pb	Cd
TEST I	8	1245.0 (7.1)	58.12 (0.53)	11.25 (0.35)
	4	601.0 (__)*	23.30 (__)*	5.80 (__)*
	2	285.2 (4.0)	11.05 (0.14)	2.63 (0.25)
	1	141.0 (2.4)	5.30 (0.21)	1.28 (0.04)
	C	<. (__)	<. (__)	<. (__)
TEST II	8	1323.0 (72.1)	58.88 (0.53)	11.63 (0.53)
	4	680.5 (23.3)	23.85 (0.92)	6.80 (0.07)
	2	314.7 (14.1)	10.60 (0.78)	3.48 (0.04)
	1	149.2 (17.2)	4.50 (0.42)	1.78 (0.00)
	C	<..(__)*	<. (__)	<. (__)
TEST III	8	1478.8 (158.4)	60.63 (10.08)	12.00 (--)*
	4	674.8 (19.6)	22.70 (0.78)	6.13 (0.04)
	2	404.0 (22.6)	12.68 (0.60)	3.75 (0.14)
	1	186.0 (__)*	7.00 (0.42)	1.90 (0.55)
	C	<. (__)	<. (__)	<. (__)
TEST IV	8	1286.6 (132.1)	55.50 (3.54)	10.75 (0.35)
	4	708.2 (6.4)	25.05 (2.62)	6.35 (0.07)
	2	360.8 (16.3)	14.50 (2.19)	3.77 (0.25)
	1	200.7 (__)*	7.00 (0.28)	1.68 (0.39)
	C	<. (__)	<. (__)	<. (__)
TEST V	8	1555.5 (158.5)	56.00 (5.66)	11.87 (0.53)
	4	762.8 (52.4)	27.53 (1.59)	6.38 (0.53)
	2	424.2 (50.0)	14.73 (1.45)	3.70 (0.21)
	1	186.7 (2.0)	6.42 (0.39)	1.85 (0.00)
	C	<. (__)	<. (__)	<. (__)
TEST VI	8	1473.2 (9.2)	63.62 (2.30)	12.63 (0.18)
	4	789.0 (142.2)	25.38 (3.50)	6.73 (0.18)
	2	399.7 (31.5)	15.00 (0.64)	3.80 (0.07)
	1	213.0 (8.6)	8.30 (0.00)	2.18 (0.04)
	C	7.2 (5.5)	<. (__)	<. (__)

Appendix Table 4 (continued).

	Treatment	Average Maximum Concentration		
	(P Units)	Cu	Pb	Cd
	8	1146.7 (23.8)	31.50 (0.71)	8.80 (0.14)
TEST VII	4	557.6 (18.2)	13.48 (0.18)	3.83 (0.04)
	2	291.3 (8.1)	7.25 (0.64)	2.05 (0.07)
	1	190.5 (39.6)	3.53 (0.04)	1.10 (0.00)
	C	6.0 (0.3)	<. (_)	<. (_)
	8	1146.9 (40.1)	31.75 (5.66)	8.23 (0.67)
TEST VIII	4	578.2 (16.3)	14.30 (0.07)	4.08 (0.11)
	2	286.6 (3.3)	7.00 (0.00)	1.97 (0.18)
	1	145.7 (0.1)	2.98 (0.32)	0.95 (0.00)
	C	11.2 (0.0)	<. (_)	<. (_)

* indicates sample size = 1.

Appendix Table 5. Concentrations of metals (P Units, see text) that cause significant survival reductions (expressed as cumulative percent survival) for brown and rainbow trout fry following the eight hour pulsed exposures. The nominal 1P concentrations (μg/L) are as follows: 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Nominal levels of hardness, alkalinity, and pH for each test expected prior to and following (Pre/Post) and during the pulse (Pulse) are shown. nP refers to the dilution of the above metals concentration where survival was reduced significantly from the respective control survival. Numbers in parentheses refer to the mean percent survival from the respective dilution and species for each test. Changes in hardness and pH during a test are symbolized as: c = constant, ↓ = depressed, and ↑↑ = highly elevated. Also see Appendix Table 6.

Test	Hardness (ppm CaCO$_3$)		Alkalinity (ppm CaCO$_3$)		pH		Hatchery brown trout	Hatchery rainbow trout	Clark Fork brown trout
	Pre/Post	Pulse	Pre/Post	Pulse	Pre/Post	Pulse			
I c Hardness c pH	100	100	100	100	7.2-8.0	7.2-8.0	4P,8P (27, 0)	4P,8P (63, 0)	
II ↓ Hardness c pH	100	50	100	50	7.2-8.0	7.2-8.0	2P,4P,8P (18, 0, 0)	1P,2P,4P,8P (91, 0, 0, 0)	
III ↓ Hardness ↓ pH	100	50	100	0	7.2-8.0	4.5	2P,4P,8P (73, 10, 0)	1P,2P,4P,8P (73, 10, 0, 0)	
IV ↓↓ Hardness ↓ pH	200	100	180	0	7.2-8.0	4.5	4P,8P (70, 0)	1P,2P,4P,8P (92, 32, 0, 0)	4P,8P (73, 0)
V ↓↓ Hardness ↓ pH	200	100	180	0	7.2-8.0	4.5	4P,8P (27, 0)	2P,4P,8P (67, 0, 0)	8P (8)
VI ↑↑ Hardness ↓ pH	200	400	180	0	7.2-8.0	4.5	no sig. mortality	8P (62)	

Appendix Table 6. Post-pulse survival analyses from the different pulsed exposures (Tests I - VI) for the Control and each treatment (1P, 2P, 4P and 8P) to fry brown and rainbow trout (hatchery brown trout, H-BNT, hatchery rainbow trout, H-RBT, and Clark Fork brown trout, CF-BNT. N is the number of replicates, Mean Survival represents the survival proportion at the end of the test, and probability, P-value, is from the Dunnett's procedure performed on arcsine transformed survival proportions. P-values less than 0.05, indicated by *, are considered to represent significantly reduced survival in the treatment from the control.

Test & Species	Treatment	N	Mean Survival	P-value $\alpha = 0.05$
Test I: H-BNT				
(constant	Control	6	0.93	
hardness & pH)	1P	6	0.98	0.479
	2P	6	0.88	0.472
	4P	6	0.27	0.001 *
	8P	6	0.00	0.001 *
Test I: H-RBT				
(constant	Control	3	1.00	
hardness & pH)	1P	3	0.97	0.463
	2P	3	0.97	0.463
	4P	3	0.63	0.001 *
	8P	3	0.00	0.001 *
Test II: H-BNT				
(depressed hardness,	Control	8	1.00	
constant pH)	1P	8	0.99	0.484
	2P	8	0.18	0.001 *
	4P	8	0.00	0.001 *
	8P	8	0.00	0.001 *
Test II: H-RBT				
(depressed hardness,	Control	7	0.99	
constant pH)	1P	7	0.91	0.012 *
	2P	7	0.00	0.001 *
	4P	7	0.00	0.001 *
	8P	7	0.00	0.001 *

Appendix Table 6 (continued).

Test & Species	Treatment	N	Mean Survival	P-value $\alpha = 0.05$
Test III: H-BNT				
(depressed	Control	8	1.00	
hardness & pH)	1P	8	0.98	0.479
	2P	8	0.73	0.001 *
	4P	8	0.10	0.001 *
	8P	8	0.00	0.001 *
Test III: H-RBT				
(depressed	Control	7	1.00	
hardness & pH)	1P	7	0.73	0.001 *
	2P	7	0.10	0.001 *
	4P	7	0.00	0.001 *
	8P	7	0.00	0.001 *
Test IV: H-BNT				
(highly depressed	Control	5	1.00	
hardness, depressed pH)	1P	5	1.00	0.500
	2P	5	0.94	0.274
	4P	5	0.70	0.001 *
	8P	5	0.00	0.001 *
Test IV: H-RBT				
(highly depressed	Control	5	1.00	
hardness, depressed pH)	1P	5	0.92	0.004 *
	2P	5	0.32	0.001 *
	4P	5	0.00	0.001 *
	8P	5	0.00	0.001 *
Test IV: CF-BNT				
(highly depressed	Control	3	0.90	
hardness, depressed pH)	1P	3	1.00	0.005
	2P	3	0.96	0.038
	4P	3	0.73	0.001 *
	8P	3	0.00	0.001 *

Appendix Table 6 (continued).

Test & Species	Treatment	N	Mean Survival	P-value $\alpha = 0.05$
Test V: H-BNT				
(highly depressed	Control	3	1.00	
hardness, depressed pH)	1P	3	0.97	0.493
	2P	3	0.87	0.232
	4P	3	0.27	0.001 *
	8P	3	0.00	0.001 *
Test V: H-RBT				
(highly depressed	Control	3	1.00	
hardness, depressed pH)	1P	3	0.97	0.395
	2P	3	0.67	0.001 *
	4P	3	0.00	0.001 *
	8P	3	0.00	0.001 *
Test V: CF-BNT				
(highly depressed	Control	3	1.00	
hardness, depressed pH)	1P	3	1.00	0.500
	2P	3	0.92	0.484
	4P	3	0.67	0.178
	8P	3	0.08	0.003 *
Test VI: H-BNT				
(highly elevated	Control	5	1.00	
hardness, depressed pH)	1P	5	1.00	0.500
	2P	5	0.96	0.493
	4P	5	0.90	0.401
	8P	5	0.76	0.095
Test VI: H-RBT				
(highly elevated	Control	5	1.00	
hardness, depressed pH)	1P	5	0.98	0.497
	2P	5	0.98	0.497
	4P	5	0.96	0.470
	8P	5	0.62	0.001 *

Appendix Table 7. Post-Pulse survival analyses from combined pulsed exposures (Tests VII and VIII) for the Control and each treatment (1P, 2P, 4P and 8P) to fry and juvenile brown and rainbow trout (hatchery brown trout, H-BNT, hatchery rainbow trout, H-RBT). N is the number of replicates, Mean Survival represents the survival proportion at the end of the tests, and probability, P-value, is from the Dunnett's procedure performed on arcsine transformed survival proportions. P-values less than 0.05, indicated by *, are considered to represent significantly reduced survival in the treatment from the control.

Species/Age	Treatment	N	Mean Survival	P-value $\alpha = 0.05$
H-BNT Fry				
(highly depressed	Control	2	1.00	
hardness, depressed pH)	1P	2	1.00	0.500
	2P	2	1.00	0.500
	4P	2	0.55	0.001 *
	8P	2	0.15	0.001 *
H-BNT Juvenile				
(highly depressed	Control	2	1.00	
hardness, depressed pH)	1P	2	1.00	0.500
	2P	2	1.00	0.500
	4P	2	0.95	0.397
	8P	2	0.60	0.007 *
H-RBT Fry				
(highly depressed	Control	2	1.00	
hardness, depressed pH)	1P	2	0.95	0.486
	2P	2	0.95	0.486
	4P	2	0.20	0.008 *
	8P	2	0.00	0.003 *
H-RBT Juvenile				
(highly depressed	Control	2	1.00	
hardness, depressed pH)	1P	2	1.00	0.500
	2P	2	1.00	0.500
	4P	2	0.05	0.001 *
	8P	2	0.00	0.001 *

FINAL REPORT

Influence of Acclimation/Adaptation on Toxicity:

Differential Tolerance and Resistance of Brown and Rainbow Trout to Water-borne Metal
Concentrations Typical of the Clark Fork River

Research Report on Injury Determination

Fishery Protocol #5

Assessment Plan, Part I

Clark Fork River Basin NPL Sites, Montana

Submitted by:

Harold L. Bergman

Red Buttes Environmental Biology Laboratory

University of Wyoming

Laramie, Wyoming 82071

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INTRODUCTION

Fish from the Clark Fork River in western Montana have been exposed to elevated concentrations of hazardous substances (metals) through direct exposure to contaminated surface water and sediments (Johnson and Schmidt 1988). Copper, Zn, Pb, and Cd are the hazardous substances most frequently elevated above water quality criteria in the Clark Fork River (USEPA 1987; USGS 1989; Lambing 1991; Moore et al. 1991). These metals are classified as "very toxic and relatively accessible" and are among the metals of highest concern for toxic effects on fish (Förstner and Wittman 1983; Baudo 1987; Heath 1987).

The primary objectives of this study as specified in the Assessment Plan (Montana 1992) were to determine whether differential sensitivity to metals toxicity exists between (1) brown and rainbow trout, and (2) resident brown trout in the Clark Fork River and control (hatchery) brown trout. Objective (1) requires evaluating the relative sensitivity of brown trout and rainbow trout to metal concentrations typical of the Clark Fork River, and Objective (2) requires evaluating whether the brown trout population from the Clark Fork River are genetically adapted through increased tolerance (i.e., higher LC50) or increased resistance (i.e., longer LT50 after acclimation to ambient metal concentrations) to elevated metal concentrations as compared to hatchery brown trout.

METHODS

Procedures employed for the experiments were those specified under Research Plans, Section 7.4.4, in the Assessment Plan: Part I, Clark Fork River NPL Sites, Montana (Montana 1992, hereafter referred to as the Assessment Plan), except where noted.

Experimental Fish

Hatchery rainbow and brown trout were obtained during the summer of 1992 from Dan Speas Fish Hatchery and from the Dubois Fish Hatchery, respectively; both hatcheries are operated by the Wyoming Game and Fish Department. Brown trout were obtained in the fall of 1991 and the spring of 1992 from the upper Clark Fork River system by fisheries biologists of the Montana Department of Fish, Wildlife and Parks [Note: Brown trout from the Big Hole River were not included in these experiments, although originally proposed in the Assessment Plan]. All fish were transported within 12 hours of collection and held at the Red Buttes Environmental Biology Laboratory, University of Wyoming. Fish were acclimated to laboratory conditions for several months, maintained, and handled according to the Assessment Plan; during this period fish were monitored daily and remained in good condition free of disease. During holding and acclimation, fish were fed 2% of body weight daily with vitamin-fortified commercial trout diet [Note: This is the recommended feeding ration specified for juvenile salmonids at 10 °C (Piper et al. 1986) and was used rather than the 5% of body weight per day specified in the Assessment Plan]. Fish were not fed 24 hours prior to and during experiments. Photoperiod was maintained to simulate natural light cycles throughout the study period. Juvenile fish were used for all experiments, and for some experiments, fish were cold-branded (Mighell 1969) to identify different treatment groups.

Exposure Water

Test water dilutions for the LC50 determinations (to assess relative metal tolerance of hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout) contained 5.0P, 2.5P, 1.2P, 0.6P, and 0.3P metal concentrations, where the nominal 1P mixture = 230 μg/L

Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. [Note: For the experiments in this report, all of the metal mixtures are expressed in units of "P" for ease of comparison with the other acute lethality experiments in the Assessment Plan (Fishery Protocol #3, Acute Toxicity in Pulse Events), rather than in units of "X", which was used in the Assessment Plan]. Metal concentrations in the 1P reference mixture are a conservative representation of the metal concentrations that have been measured during episodic pulse events and during "redwater" discharge events documented in the Clark Fork River from as early as 1960 and before and as recently as 1991 (see Surface Water Resources Chapter). The specific metal concentrations (in μg/L) selected for Zn, Cu and Cd in the 1P reference metal mixture, 230 Zn, 120 Cu and 2.0 Cd, were the dissolved concentrations actually measured in the Clark Fork River at Deer Lodge during a fish kill on July 12, 1989 (Table 1); because dissolved Pb concentrations were below detection limits for this particular pulse sample, the Pb concentration for the 1P reference pulse was set at 3.2 μg/L, which is the chronic water quality criterion at water hardness of 100 mg/L $CaCO_3$ (USEPA 1987). This set of metal concentrations for the reference (1P) pulse metal mixture used in laboratory experiments is conservative, given the remarkably high total recoverable metal concentrations measured at different locations and times during storm pulse or "redwater" discharge events documented on the upper Clark Fork River (see Table 1 for a summary).

During the acclimation period for the LT50 and mean time to death determinations (to assess the relative ability of hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout to acclimate to a metals mixture), test water exposures contained either 0.0P metals (control) or 0.2 dilution of the 1P metals mixture, where 0.2P = 46 μg/L Zn, 24 μg/L Cu, 0.64 μg/L Pb, and 0.40 μg/L Cd and where the 0.2P metal concentrations are within the range of base flow conditions measured in the Clark Fork River (Lambing 1991). During the challenge periods, fish were exposed to a 1P metals mixture as specified above.

Experimental Design: LC50 Determination

Fish were acclimated for a minimum of one month to control water conditions

(hardness, 100 mg/L $CaCO_3$; alkalinity, 80 - 110 mg/L $CaCO_3$; pH, 7.2 - 7.8; temperature, 10 °C). Individuals of each species or strain (hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout) were randomly allocated to treatments. Each exposure treatment (5.0P, 1.2P, 0.6P, 0.3P and 0.0P-control) was duplicated and each duplicate treatment included 40 individuals for each species/strain.

Mortality (defined as cessation of opercular movement) was monitored every two hours for the first twelve hours and every six hours for the remainder of the experiment. Dead fish were removed immediately, and length and weight of every individual was recorded (Appendix Table 1).

Experimental Design: LT50 and Mean Time to Death Determinations

Fish were held for a minimum of one month in control water (hardness, 100 mg/L $CaCO_3$; alkalinity, 80 - 110 mg/L $CaCO_3$; pH, 7.2 - 7.8; temperature, 10 °C). Cold-branded individuals of each species/strain (hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout) were transferred in groups of 20 from holding tanks to either acclimation (0.2P) or control (0.0P) tanks. At the onset of the acclimation exposure, 140 individuals/exposure of hatchery rainbow trout and hatchery brown trout were used and 75 individuals/exposure of Clark Fork brown trout were used. For each LT50 determination, in each of the challenge periods described below, 30 individuals from each of the 0.2P and 0.0P acclimation groups were transferred to the challenge tanks containing 1P metals concentration, combining both 0.2P and 0.0P acclimated groups (with distinctive cold-branding marks) of a given species/strain into a single challenge tank.

Figure 1 illustrates the time course for the acclimation treatments preceding each challenge period. Challenge I followed three weeks in the 0.2P or 0.0P acclimation treatments. Challenge II followed five weeks of acclimation to the 0.2P or 0.0P acclimation treatments. Challenge III (using only hatchery species) followed six weeks of acclimation to the 0.2P acclimation treatment and then followed by two weeks of de-acclimation in 0.0P treatment (control) for the "metals acclimated" group, and eight weeks of acclimation to the

0.0P treatment for the "control or non-metals acclimated" group. [Note: In the Assessment Plan, only Challenges I (3 weeks acclimation) and II (5 weeks acclimation) were described. Since sufficient numbers of hatchery brown and rainbow trout (but not Clark Fork brown trout) remained at the end of the Challenge II acclimation period, Challenge III was added to the experimental design to determine whether acclimation was lost following an additional 2 weeks in control water].

During each challenge, mortality (defined as cessation of opercular movement) was monitored every two hours for the first twelve hours and every six hours for the remainder of the experiment. Dead fish were removed immediately, and length and weight of every individual was recorded (Appendix Table 2).

Chemical Analyses of Water

Test and control waters were analyzed for metals, hardness, alkalinity, pH, and dissolved oxygen each day during the LC50 and LT50 determinations and every three days during the acclimation exposures.

Statistical Analyses

To evaluate relative sensitivity of the test fish to the metals mixture, LC50 values and 95% confidence intervals were computed using the Spearman-Karber method (Hamilton et al. 1977). LC50 values were computed using average exposure concentrations (μg/L Zn, Cu, Pb and Cd) analyzed by atomic absorption spectroscopy. For comparison, the Zn-LC50 values and confidence limits were converted into "P" units. The Zn-LC50 estimates were determined to be significantly different if the 95% confidence intervals did not overlap. This is analogous to a one-tailed test of significant differences at $\alpha = 0.05$ with overall protection of $(0.95)^3$.

Results from the acclimation experiments to determine relative resistance to metals exposure were analyzed using two different statistical procedures: (1) estimates of the "median lethal time" (LT50) and, (2) estimates of the "mean time to death". LT50 estimates and 95%

confidence intervals were computed using the jackknife procedure (Manley 1991). To compare the mean time to death (in hours) for the three species exposed to the two different acclimation levels (0.0P and 0.2P), two-way Analysis of Variance (ANOVA) was used. Challenges I and II included three levels of the species factor, hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout, and two levels for the acclimation factor, 0.0P for the control acclimation water, and 0.2P for the metals acclimation water. Challenge III included two levels of the species factor (the two hatchery species) and two levels for the acclimation factor, 0.0P for the control acclimation water, and "0.2P→0.0P" for the acclimation exposure that initially contained 0.2P metals and then was switched to control water, 0.0P, for the de-acclimation period. The response variable measured on each fish was the number of hours until death, where the value of this response was the midpoint of the interval between the discrete time period where the fish was observed dead and the previous time period observed. A level of $\alpha=0.10$ was used to determine significant effects in the ANOVA. For each of the challenge periods, a series of t-tests (Montgomery 1983) were used to: (1) compare (*a priori*) the mean time to death between acclimation levels within each species, and (2) compare (*a priori*) the mean time to death between species within each acclimation level. Satterthwaite's correction on the degrees of freedom was employed in all t-tests when the null hypothesis of equal variances was rejected using the folded F test (Steel and Torrie 1980) at the $\alpha=0.01$ level. Differences were judged to be significant when t-test P-values were below the conservative Bonferonni adjusted value (Manley 1991) of $\alpha=0.10/9=0.0111$ for Challenge I and II contrasts and $\alpha=0.10/4=0.025$ for Challenge III contrasts.

RESULTS

Water Chemistry

Measured hardness, alkalinity, and pH values were within 6% of nominal in all tests with the exception of water hardness during the LC50 determination, which averaged only 1.5% over nominal but ranged as much as 11% over nominal (Appendix Table 3). Dissolved oxygen concentration was between 90 - 98% saturation and temperature remained at 10.0 ± 0.5 °C in all exposure tanks during all tests (Appendix Table 4).

Average measured Zn concentrations were 95 - 104% of the nominal concentrations from the LC50 determination and were 108 - 125% of the nominal concentrations from the acclimation and LT50/mean time to death determinations. Average Zn, Cu, Pb, and Cd concentrations and standard deviations for the different exposure dilutions are summarized in Appendix Table 5.

LC50 Estimates

Survival was 100% for all control fish during the LC50 determinations (Figure 2). In the highest exposure concentration (5.0P), the cumulative mortalities were essentially equal for all three species/strains; mortalities were observed as early as eight hours and survival was at or near 0% at 12 hours for all three species/strains (Figure 2). Also, metal concentrations as low as 0.6P were lethal to hatchery and Clark Fork brown trout after 30 hours, and hatchery rainbow trout after 42 hours.

The LC50 values for hatchery and Clark Fork brown trout were similar at each time interval except at 96 hours, when the LC50 for Clark Fork brown trout was significantly lower than for hatchery brown trout (Figure 3). Clark Fork brown trout had significantly lower LC50's than hatchery rainbow trout at 24, 48, 72, and 96 hour. Hatchery brown trout had significantly lower LC50's than hatchery rainbow trout at 48, 72, and 96 hour ($\alpha = 0.05$; Figure 3 and Appendix Table 6). Both hatchery brown trout and Clark Fork brown trout LC50 point estimates were below the 1P metals concentration by 48 hours, and the hatchery rainbow trout LC50 point estimate was below 1P by 72 hours (Figure 3 and Appendix Table

6). Overall, at 96 hours the relative tolerance of the three species/strains was hatchery rainbow trout > hatchery brown trout > Clark Fork brown trout, and all differences were significant ($\alpha = 0.05$).

LT50 and Mean Time to Death Estimates

Survival was 100% in both the 0.2P and 0.0P acclimation exposures for both hatchery and Clark Fork brown trout, and 98% for hatchery rainbow trout in both the 0.2P and 0.0P acclimation exposures throughout the entire acclimation periods. Cumulative survival/mortality plots (Figure 4) show a marked increase in survival time when fish are acclimated to 0.2P as compared to 0.0P, and the increase in survival time is substantially greater for both hatchery and Clark Fork brown trout after five weeks of acclimation than after three weeks of acclimation.

LT50 estimates calculated from these data for Challenge I (3-week acclimation) were significantly longer for all three species/strains when acclimated at 0.2P metals concentration as compared to 0.0P controls, and Clark Fork brown trout had a significantly longer LT50 than either hatchery brown or rainbow trout when all were acclimated to 0.2P metals (these observations are based on comparisons of 95% confidence limits as presented in Figure 5 and Appendix Table 7).

For Challenge II (5-week acclimation) the LT50 was significantly longer for Clark Fork brown trout acclimated to 0.2P metals than for 0.0P controls, and the Clark Fork LT50 was also significantly longer than hatchery rainbow trout, but not hatchery brown trout, when all fish were acclimated to 0.2P metals for 5 weeks (Figure 5 and Appendix Table 7). In this analysis, however, hatchery rainbow trout LT50's were not different after 5 weeks acclimation in 0.2P metals as compared to 0.0P controls, and although hatchery brown trout had a longer LT50 after 0.2P acclimation than after 0.0P control acclimation, no statistical inference could be drawn because confidence limits could not be calculated for the 0.0P control fish due to low variance (Figure 5 and Appendix Table 7).

The LT50 estimate from Challenge III for 0.2P→0.0P ("de-acclimated") hatchery

brown trout was significantly less than that of the 0.0P hatchery brown trout, while the LT50 estimate for 0.2P→0.0P hatchery rainbow trout was significantly longer than that of the 0.0P hatchery rainbow trout (Figure 5 and Appendix Table 7).

Similar results comparing acclimation effects and differences among species are demonstrated by the ANOVA analyses of results for mean time to death as were obtained in the estimation of LT50 values, and are presented to augment the traditional LT50 evaluation shown above. Figure 6 and Appendix Tables 8a, 9a, and 10a show the mean time to death from Challenges I, II, and III, respectively. For all three challenges, the species x acclimation interaction was significant (P-value < 0.0001; Appendix Tables 8b, 9b, and 10b). Therefore, main effects in the overall ANOVA are not easily interpreted. Rather, comparisons were only interpreted for relevant pairs of mean values using *a priori* *t*-tests (Appendix Tables 8c, 9c, and 10c).

In Challenge I, all species/strains had a significantly longer mean time to death after three weeks of 0.2P acclimation than after three weeks of control 0.0P acclimation (Figure 6 and Appendix Table 8c). Although mean time to death was significantly longer for Clark Fork brown trout and hatchery rainbow trout than for hatchery brown trout when acclimated to 0.0P control water, Clark Fork brown trout also had a significantly longer mean time to death than either hatchery brown or hatchery rainbow trout when acclimated to 0.2P metals (Figure 6 and Appendix Table 8c). Hatchery brown trout and hatchery rainbow trout did not have significantly different mean times to death in the 0.2P acclimation but were different in the 0.0P acclimation (Figure 6 and Appendix Table 8c).

In Challenge II, all species/strains had a significantly longer mean time to death after five weeks of 0.2P acclimation than control fish from the 0.0P acclimation (Figure 6 and Appendix Table 9c). Although Clark Fork brown trout had a significantly longer mean time to death than either the hatchery browns or rainbows when acclimated to 0.0P control water, Clark Fork brown trout also had a significantly longer mean time to death than either hatchery brown or hatchery rainbow trout when acclimated to 0.2P metals (Figure 6 and Appendix Table 9c). Hatchery brown trout also had a significantly longer mean time to death than

hatchery rainbow trout when acclimated to 0.2P metals (Figure 6 and Appendix Table 9c).

In Challenge III, after two weeks of de-acclimation, hatchery brown trout lost their acclimation to the metals mixture, and were apparently even sensitized to the metals mixture, as demonstrated by the significantly shorter mean time to death for the 0.2P→0.0P group than the 0.0P control group. Hatchery rainbow trout, on the other hand, had a significantly longer mean time to death in the 0.2P→0.0P acclimation than in the 0.0P acclimation. Hatchery rainbow trout also had a significantly longer mean time to death than hatchery brown trout in the 0.2P→0.0P acclimated group (Figure 6 and Appendix Table 10c).

Although the results presented above and in Figure 6 illustrate significant acclimation (i.e., increased mean time to death) for both hatchery and Clark Fork brown trout, with the effect significantly greater in Clark Fork browns than hatchery browns, part of this effect in Challenge II may have been caused by differential increases in fish size (weight; see Appendix Table 2) through the course of the acclimation experiments. Note, for instance, the significantly greater mean time to death in Clark Fork browns, as compared to hatchery browns, even in the control 0.0P acclimated fish (Figure 6). In spite of this possible influence of fish size on the acclimation effect in comparing the two brown trout strains, the substantially and significantly greater mean time to death in metal acclimated brown trout than in similarly acclimated rainbow trout is clear (Challenge II in Figure 6).

DISCUSSION

Acute Lethality of Clark Fork Metal Concentrations

Survival of both brown trout and rainbow trout was markedly reduced in this laboratory study when the fish were acutely exposed to a metal mixture containing Zn, Cu, Pb and Cd within the range of concentrations observed during fish kill events in the Clark Fork River. For instance, the 48-, 72- and 96-hour LC50 values shown in Figure 3 for hatchery and Clark Fork brown trout and hatchery rainbow trout were at or well below the 1P reference metal concentrations of (in μg/L) 230 Zn, 120 Cu, 3.2 Pb and 2.0 Cd.

As described earlier in the Methods section, the 1P metals mixture represents the dissolved metal concentrations measured in the Clark Fork River near Deer Lodge during the major fish kill documented on July 12, 1989. In comparing the toxicity of this metal mixture in the laboratory studies reported here and the field conditions reported for the July 12, 1989, fish kill, two factors would tend to cause the laboratory studies to overestimate the toxic conditions during this fish kill, and two factors would tend to cause the laboratory results to underestimate the toxic conditions that obtained during the fish kill. Factors that would overestimate toxicity in the laboratory studies are (1) water hardness in the laboratory was held at 100 mg/L $CaCO_3$ whereas hardness measured at Deer Lodge during the fish kill was 200 mg/L $CaCO_3$, which would make the metal mixture somewhat more toxic in the laboratory studies, and (2) the exposure duration may have been shorter during the fish kill of July 12 than the 48-hour time period required for a 1P metal mixture to cause substantial mortality (50%) in the laboratory (though the length of the July 12 pulse is not well documented, and may have been shorter or longer than 48 hours). Factors that would underestimate toxicity in the laboratory as compared to field conditions during the July 12, 1989, fish kill include (1) the metal concentrations were no doubt considerably higher upstream from Deer Lodge in the Clark Fork River than those measured at Deer Lodge on July 12 (i.e., though dissolved measurements are not available, the total recoverable concentrations for Zn and Cu were 14,000 and 13,300 μg/L, respectively, upstream at the Mill-Willow Bypass during the same fish kill event (Table 1)), and (2) the laboratory results are reported here as discrete time (i.e.,

48-hour) median lethal concentrations or LC50's, to facilitate the most robust statistical and toxicological comparison of species/strain sensitivities. But metal concentrations that would cause a substantial fish kill in the field could be as low as the "LC1" or "LC10" concentration (where we would expect 1% and 10%, respectively, of a population to be killed). Overall, the results reported here for controlled laboratory experiments on the acute lethality of metal mixtures, at concentrations that have been documented at equal or higher concentrations during pulse or "redwater" discharge events in the Clark Fork River, clearly support the conclusion that fish kills associated with these events were caused by toxic metal concentrations.

Previously published laboratory and field studies have demonstrated adverse effects of metals such as Zn, Cu, Pb, and Cd on survival of salmonids and other freshwater organisms (Nehring and Goettl 1974; Finlayson and Verrue 1982; Roch and McCarter 1984a; Todd et al. 1984; Moore et al. 1991). For example, chinook salmon exposed to a metal mixture similar to that in the present study (except that Pb was not included), had 96-hour LC50s for mixture constituents of 121, 37 and 1.1 μg/L for Zn, Cu and Cd, respectively (hardness, 21 mg/L $CaCO_3$; pH 7.0 - 7.3; temperature 11 - 13 °C; Finlayson and Verrue 1982). In spite of higher water hardness and pH values in the present study, the metal concentrations in the chinook salmon experiments were very similar to the metal concentrations at the 96-hour LC50 dilution found here, where the Zn, Cu and Cd concentrations (in μg/L) were, respectively, 132, 83 and 1.4 for hatchery brown trout, 97, 78 and 1.3 for Clark Fork brown trout, and 182, 127 and 2.1 for hatchery rainbow trout (Appendix Table 6). Compared to chinook salmon, hatchery brown trout and Clark Fork brown trout had similar Zn and Cd but somewhat higher Cu concentrations at the LC50, whereas hatchery rainbow trout had higher concentrations of all three metals at the LC50 dilution. Several additional conclusions are warranted from this comparison. First, the sensitivity of brown trout to this metal mixture appears to be very similar to that of chinook salmon, whereas rainbow trout appear to be somewhat more tolerant to the metal mixture than are chinook salmon. And second, the very close similarity of Finlayson and Verrue's results with chinook salmon to those reported here for brown and rainbow trout constitutes an independent validation of our results on the acute

lethality of this metal mixture to trout.

Acclimation to Metal Concentrations Typical of the Clark Fork

Acclimation to a metals mixture at concentrations typical of base flow conditions in the Clark Fork River (0.2P metals mixture) significantly increased the resistance of all three species/strains during a challenge exposure to a 1P metals mixture, as demonstrated by increases in LT50's and mean times to death for metal-acclimated fish in Figures 5 and 6. In both hatchery and Clark Fork brown trout, this acclimation effect on resistance to the 1P metals challenge was dramatically greater after five weeks of metals acclimation as compared to only three weeks of acclimation (e.g., mean time to death for Clark Fork brown trout increased from 26 or 50 hrs in control (0.0P) acclimations, to 66 hrs after three weeks metals acclimation, and to 245 hrs after five weeks metals acclimation; see Figure 6). Moreover, the increase in resistance was significantly greater for Clark Fork brown trout than for hatchery brown trout after both three and five weeks of metals acclimation, although this difference may have been attributable in part to differences in fish size between the two strains, as noted in the Results section. In spite of this dramatic acclimation response in brown trout, the increase in resistance was completely lost in hatchery browns after two weeks of de-acclimation; in fact, the hatchery browns appear to be sensitized to the challenge metals mixture after de-acclimation as demonstrated by significantly <u>shorter</u> mean time to death for this group shown in Figure 6 (Clark Fork browns could not be tested in the Challenge III de-acclimation experiment because insufficient numbers of fish were available).

Although hatchery rainbow trout also acclimated to the metals mixture, as demonstrated by the significantly increased mean time to death for the 0.2P acclimated rainbows (compared to the 0.0P acclimated control fish) during both Challenge I and II, the acclimation effect at five weeks was markedly and significantly less for rainbow trout than for either brown trout strain (Figure 6). Also in contrast to brown trout, which were vulnerable to the metals challenge after de-acclimation, the rainbow trout retained their resistance even after two weeks of de-acclimation (Challenge III in Figure 6).

Many published studies have demonstrated acclimation to metals by fish, although in most of these studies single metal exposures were employed. For instance, increased resistance (e.g., increased LT50's) or increased tolerance (e.g., increased LC50's) to acutely lethal Zn concentrations has been demonstrated after pre-exposure to sublethal Zn concentrations in rainbow trout embryos (Sinley et al. 1974), rainbow trout fry (Bradley et al. 1985), rainbow trout juveniles (Lloyd 1960; Anadu et al. 1989), sockeye salmon alevins (Chapman 1978), and flagfish larvae (Spehar 1976; Spehar et al. 1978). Similar acclimation-induced increases in resistance or tolerance have been reported for various fish species pre-exposed to sublethal and then challenged to lethal concentrations of Cu (Dixon and Sprague 1981), Cd (Pascoe and Beattie 1979; Duncan and Klaverkamp 1983; Benson and Birge 1985) and aluminum (McDonald et al. 1991; Mueller et al. 1991; Wilson et al. 1993a,b). And in a study using a mixture of metals (Roch and McCarter 1984a), rainbow trout pre-exposed to a sublethal mixture of Zn, Cu and Cd showed increased tolerance of a subsequent lethal challenge to the mixture.

Reduced growth has been identified as a major cost associated with sublethal metal exposures, including sublethal exposures producing acclimation (Sprague 1985). Previous studies on brown trout (Sadler and Lynam 1987, 1988, Reader et al. 1988), brook trout (Mount et al. 1988a,b, Ingersoll et al. 1990a,b) and rainbow trout (Wilson and Wood 1992; Wilson et al. 1993a,b) demonstrated reduced growth in response to aluminum exposures. Direct measures of acclimation costs for juvenile rainbow trout acclimated to aluminum include reduced growth and reduced swimming capacity, related to a "damage/repair" phenomenon involving physiological, biochemical and structural changes at the gills (Wilson et al. 1993a).

Reduced long-term growth has been documented during sublethal exposures resulting in acclimation to Zn and Cu mixtures (Finlayson and Verrue 1980) and to Zn, Cu and Cd mixtures (Roch and McCarter 1984c; Roch and McCarter 1986) and in these studies, reduced growth was considered to be a sensitive measure of the deleterious effect/toxicity of these metal mixtures. During sublethal exposures resulting in acclimation to individual metals,

reduced growth has also been documented for Zn (Hobson and Birge 1989) and Cu (Dixon and Sprague 1981; Collvin 1984; Sprague 1985). This reduced growth associated with acclimation to metals such as Zn, Cu and Cd is routinely considered to be the "cost of acclimation" and is often tied to the specific structural (e.g., mucous cell proliferation; Wilson et al. 1993a), physiological (e.g., respiratory and ionoregulatory status; Collvin 1984; Wilson et al. 1993b) or biochemical (e.g., metallothionein induction; Sprague 1985; Hobson and Birge 1989) changes that constitute the actual mechanisms of acclimation.

Moreover, these "costs of acclimation" that can be associated with reduced growth in fish acclimated to metals can be important to consumers and managers of aquatic resources due to the potential for reduced reproductive potentials (e.g., increased egg resorption, decreased egg size, reduced spawning success; McFarlane and Franzin 1978) yielding lowered recruitment of young fish into the population (Duncan and Klaverkamp 1983). In the experiments reported here, the cost of acclimation may be indirectly demonstrated by the measured response of hatchery brown trout becoming more sensitive to the acutely lethal effects of the challenge metal mixture upon de-acclimation (i.e., in Challenge III the de-acclimated browns were less resistant than the control browns; Figure 6).

Individual and Joint Metal Contributions to Mixture Toxicities

Based on the individual metal concentrations at the 96-hour LC50 dilution for rainbow trout in this study, and 96-hour LC50 concentrations reported for the individual metals and rainbow trout, we calculated the percent contribution of each metal to the mixture 96-hour LC50, using methods described by Parkhurst et al. (1981). The determined order of toxicity/potency contributed by each individual metal to the 96-hour LC50 for rainbow trout exposed to the Clark Fork metal mixture was Cu > Zn > Cd > Pb, with Cu contributing 79.8%, Zn contributing 16.8% and Cd contributing the remaining 3.4% (Table 2). Because acute lethalities of individual metals to brown trout were not available in sufficient detail to match the water quality conditions (e.g., hardness, pH) in the present study, we were unable to complete a similar analysis for brown trout.

Previous studies, using metal mixtures with Zn:Cu:Cd ratios similar to the ratios used in the present study, have also demonstrated stronger contributions by Cu over Zn and Cd or other metals (e.g., Rehwoldt et al. 1971; Finlayson and Verrue 1982).

The metals mixture of Zn, Cu, Pb and Cd used in the experiments from the present study to represent elevated metals in the Clark Fork River was determined to be less than additive (i.e., acting antagonistically) for rainbow trout (additivity index = -0.97, Table 2). The joint toxicity of multiple metals simultaneously administered to water has been described as either additive, synergistic, or antagonistic, compared to the individual toxicity of metals. Variations in LC50s and predictions of individual toxicant's actions often depend on the particular ratio of metals present and on the chemistry of the dilution water (Roch and McCarter 1984b). For example, Zn and Cu mixtures in soft water with high metal concentrations interacted synergistically, but in hard or soft water with low metal concentrations interacted additively; deviation from Zn and Cu synergism at lower concentrations and higher hardnesses may be explained by the relative proportions of Zn, Cu, and Ca (Lloyd 1961). Mixtures of Zn, Cu, and Cd are reported additive or synergistic for fathead minnows, antagonistic for chinook salmon, and additive for rainbow trout (Eaton 1973, Finlayson and Verrue 1982, and Roch and McCarter 1984b, respectively). Combinations of Zn and Cu are reported additive for rainbow trout and Atlantic salmon (Lloyd 1961; Sprague 1964).

Whether the metals in the Clark Fork metals mixture are indeed acting additively, synergistically or antagonistically at any given time in the Clark Fork River would depend on the water quality characteristics in the river during pulses of exposure. It is clear from the above analysis that Cu and Zn are, by far, the most important metals in the Clark Fork accounting for upwards of 95% of the potency.

Relative Species/Strain Sensitivity to Clark Fork Metal Mixtures

The primary objective of this study was to determine differential sensitivity of hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout to metals exposure

typical of the Clark Fork River. To meet these objectives it was necessary to: (1) measure the relative sensitivity (i.e., determine LC50) of the different species/strains when exposed to a metals mixture typical of the Clark Fork River, and (2) measure relative resistance (i.e., determine LT50 and "mean time to death") of the different species/strains when exposed to a lethal metal mixture typical of the Clark Fork River after the fish had been acclimated to control (no metals) or chronic metal concentrations typical of baseflow conditions in the Clark Fork River. Companion studies on brown and rainbow trout, conducted under other fishery protocols in the Assessment Plan, produced additional information on relative sensitivity of fishes to metals. These included acute toxicity of pulse events (Fishery Protocol #3), chronic toxicity from food-chain exposures (Fishery Protocol #1), chronic toxicity due to physiological impairment from the food-chain exposures and from the field (Fishery Protocol #2), and behavioral avoidance responses (Fishery Protocol #4). As is evident from Table 3, brown trout are more sensitive under some conditions and visa versa.

Non-acclimated Clark Fork brown trout were most sensitive of the three species/strains tested based on LC50 determinations, using constant water quality conditions (relative sensitivity Clark Fork brown trout > hatchery brown trout > hatchery rainbow trout; Table 3). But when fish were acclimated to a 0.2P metals mixture, typical of the Clark Fork River, rainbow trout were the most sensitive and Clark Fork brown trout generally were the least sensitive to an acutely lethal challenge with a 1P metals mixture (Figures 5 and 6, Table 3).

Similarly, under acutely lethal metal pulse conditions used in Fishery Protocol #3, the relative sensitivity of the three species/strains is altered by varying water quality conditions that accompany the pulsed metal exposure. As presented in the companion report on pulsed metal exposures and summarized in Table 3, if water hardness and pH are held constant during the metal pulse, brown trout are more sensitive than rainbow trout. But under conditions that are more typical of storm pulse chemistry in the Clark Fork River (e.g., depressed hardness and pH), the relative sensitivity to the metal pulse is reversed, with rainbow trout being the most sensitive.

The relative sensitivity of rainbow and brown trout to chronic exposures at low metal concentrations was evaluated in the accompanying reports on Fishery Protocols 1, 2 and 4 on food chain, physiological impairment and behavioral avoidance, respectively, and overall conclusions are again summarized in Table 3. Because of reduced feeding activity and, possibly, other causes, rainbow trout are somewhat more sensitive to effects of dietary metals (from the Clark Fork River) on growth. But in at least three physiological/structural measurements in these experiments (gut impaction, lipid peroxidation and histology; Table 3) brown trout appear to be more sensitive than rainbow trout.

Among all of the responses to metals that were measured, the most marked difference in species sensitivity was observed with behavioral avoidance. As documented in the Fishery Protocol #4 report and summarized in Table 3, rainbow trout were substantially more sensitive than brown trout in avoiding low metal concentrations typical of even base flow conditions in the Clark Fork.

In summary, the relative sensitivity of the three species/strains varies with exposure condition and response measured. But considering the water quality conditions that prevail in the Clark Fork River, continuous low-level, chronic metal concentrations with frequent, short-duration metal excursions that are sufficiently elevated to cause substantial fish kills, rainbow trout appear most sensitive. Moreover, Clark Fork brown trout appear most resistant. Evidence that supports these conclusions includes: (1) the greater sensitivity of rainbow trout than brown trout (especially Clark Fork browns) to acute metal exposure when acclimated to lower-level metals, (2) the greater sensitivity of rainbow trout when metal pulses mimic conditions in the Clark Fork River, with depressed hardness and pH, and (3) the greater sensitivity of rainbow trout in the behavioral avoidance response to metals.

Evidence of Genetic Adaptation and Physiological Injury in the Clark Fork Brown Trout Population

In addition to the above, this study investigated whether Clark Fork metals mixture exerted physiological/genetic responses differently in brown and rainbow trout. To do this we

measured and compared tolerance and resistance limits of Clark Fork River brown trout to hatchery strains of brown and rainbow trout. While Clark Fork browns did not have higher LC50s than hatchery browns or rainbows when no acclimation was provided, they did have higher LT50s and mean time to death than hatchery browns and rainbows when acclimated to 0.2P. The higher resistance to metals of Clark Fork browns indicates that these individuals have undergone a selective pressure providing greater physiological capabilities to withstand acute metals exposures for longer periods of time.

Fish populations from polluted areas often tolerate/resist elevated hazardous substances more than populations from unpolluted areas (e.g., Benson and Birge 1985). And the hazardous substances may exert short-term/intermediate lethal effects or exert longer-term pressures that either: (1) change the physiological/genetic responses (i.e., acclimation/adaptation) and/or, (2) limit population distributions and health (i.e., avoidance/growth).

The energy demands of structural, physiological or biochemical compensation required for metals acclimatization can reduce growth (discussed above and below) and could affect food-resources demand, body condition, migration, and reproduction. Phenotypic responses to environmental perturbations, if sustained long enough, can cause selection for traits that favor survival. However, such selection may reduce genetic variability and the ability of the population to adapt to other sources of stress. The expression of greater resistance resulting from metals acclimation would, in the short-term, provide a greater ability for individuals with such phenotypes to persist in contaminated areas. But the cost for such trait expression may result in reduced fitness since exposure to hazardous substances over time should result in loss of genetic variability (Gillespie and Guttman 1988; Chagnon and Guttman 1989).

More direct evidence for reduced fitness was demonstrated by reduced growth in brown trout exposed to diet and water-borne metal mixtures representing those of the Clark Fork River (see Fishery Protocol #2, chronic toxicity from food chain exposures). Because growth rate directly relates to fecundity in teleosts (Wootton 1979), it is important for

reproductive success and should serve well to indicate population fitness. Reduced growth caused by metals exposure is likely due to additional energetic costs for maintaining homeostasis in the presence of metals. Consequently, the metabolic demands from detoxifying metals to maintain homeostatic balance injure trout populations by diverting resources from normal growth processes. Hence physiological or genetic changes permitting metals acclimation, over the long term, have costs associated that may ultimately reduce survival in the wild.

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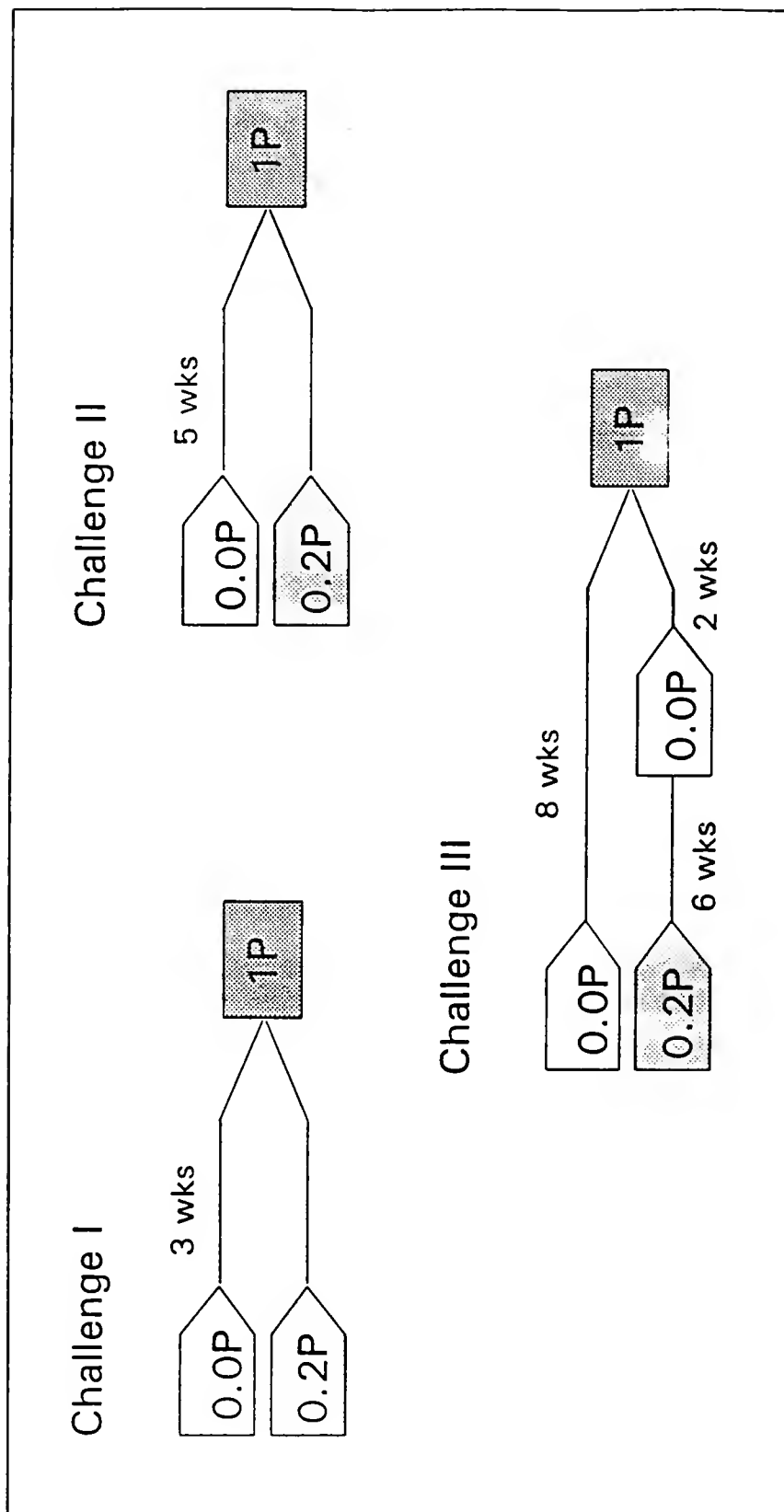
Figure 1. Schematic diagram of the three challenge periods from the LT50 and mean time to death determinations for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout acclimated to control water (0.0P) or low levels of metals (0.2P) and then challenged with the 1P metals mixture. Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd.

Figure 2. Cumulative percent survival throughout the 96 hour LC50 determinations for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout exposed to dilutions of a mixture of Zn, Cu, Pb, and Cd. Exposure dilutions contained either 0.0P-control, 5.0P, 2.5P, 1.2P, 0.6P, or 0.3P metal concentrations where the nominal metal concentrations (μg/L) in the 1P mixture = 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. P Units represent the notation for observed metal concentrations during pulse events in the Clark Fork River (see text).
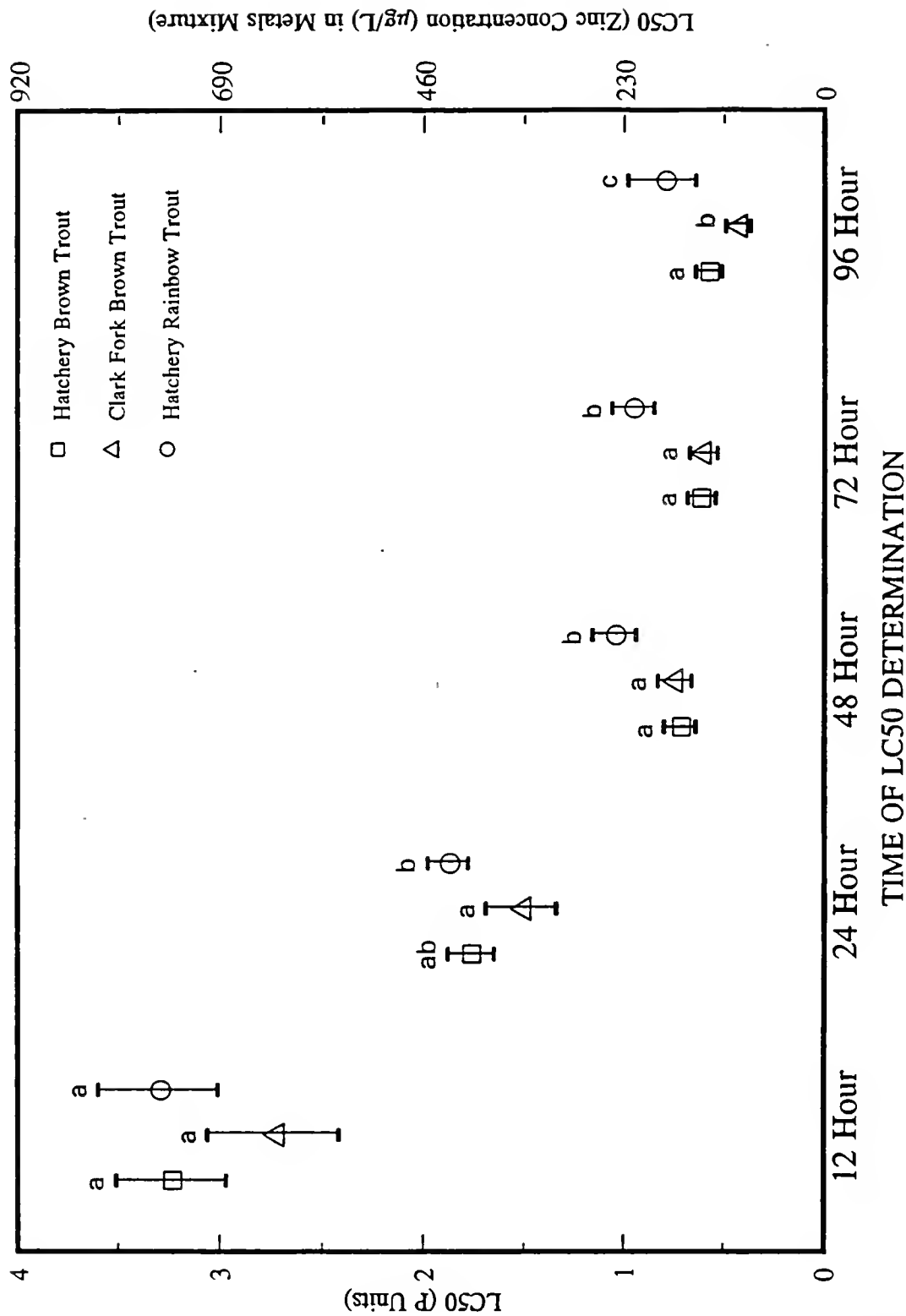
Figure 3. Median lethal concentrations (LC50) and 95% confidence intervals (P units) at 12, 24, 48, 72 and 96 hour exposure times for hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout exposed to dilutions of a mixture of Zn, Cu, Pb, and Cd. LC50 values based on the measured Zn concentrations (μg/L) and converted into P Units, where 1P = 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. LC50 calculations made using the Spearman-Karber method (Hamilton et al. 1977). For comparisons within each time period, LC50 values shown with the same letter superscript (above the upper 95% confidence limit) are not significantly different, based on comparison of 95% confidence limits ($\alpha = 0.05$) with overall protection of $(0.95)^3 = 0.86$. Also see Appendix Table 6.
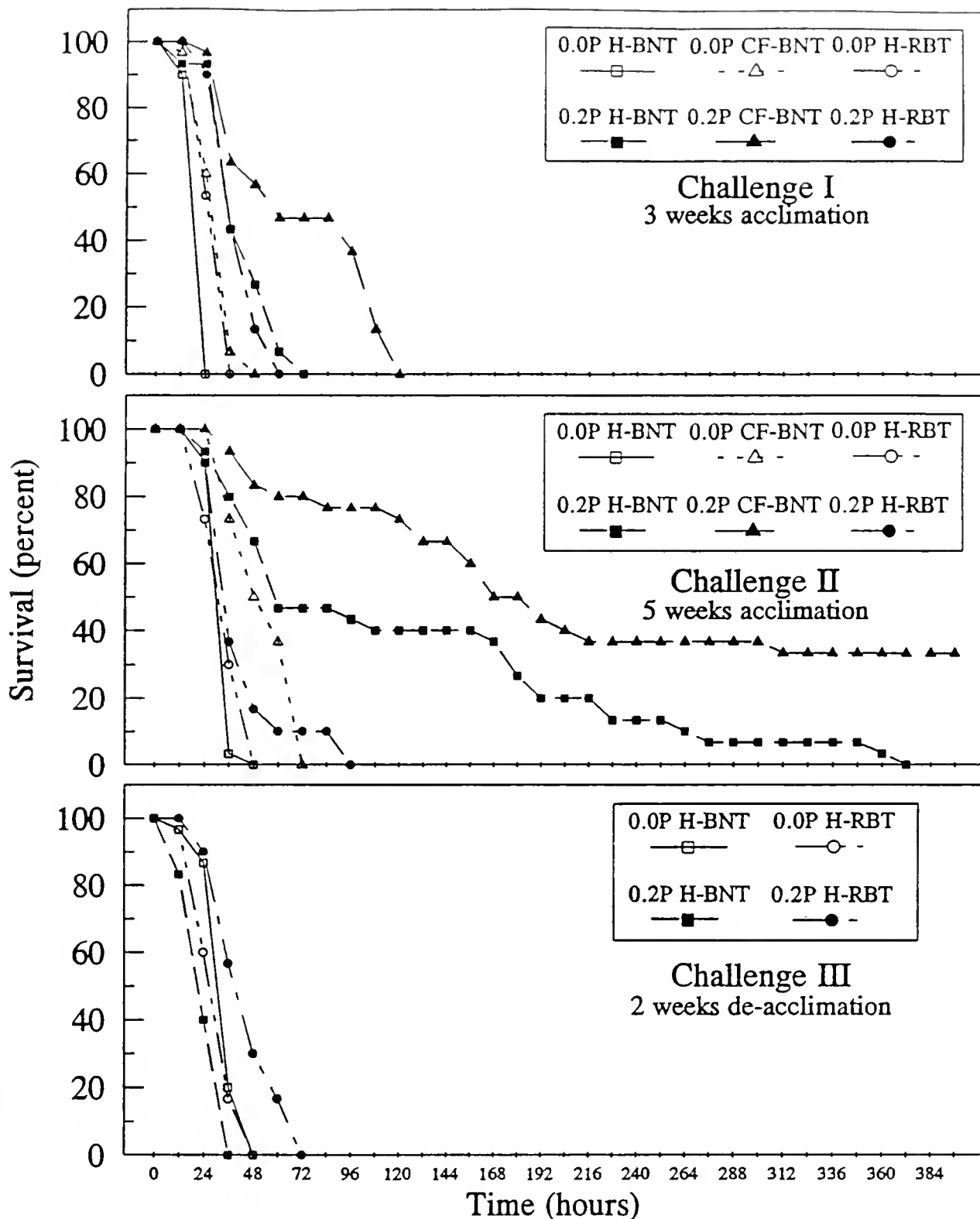
Figure 4. Cumulative percent survival during each challenge period (LT50 and mean time to death determinations) where brown and rainbow trout were challenged with a 1P exposure. Challenges I and II followed 3 and 5 weeks acclimation, respectively, to low levels of metals, 0.2P, and control water, 0.0P, for hatchery brown trout, Clark Fork brown trout, and hatchery rainbow trout. Challenge III followed 2 weeks de-acclimation in control water (0.0P) for hatchery brown and rainbow trout. Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd.

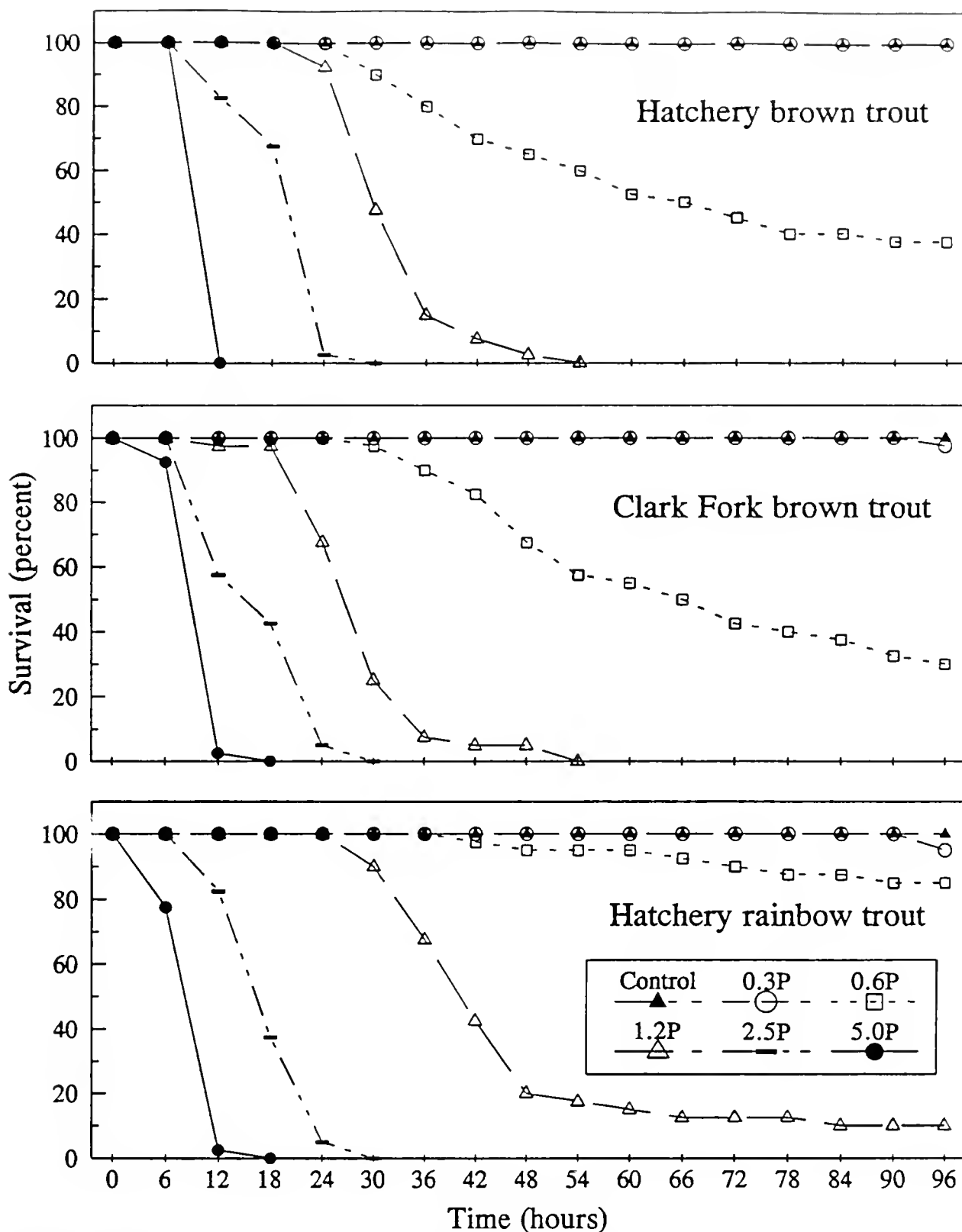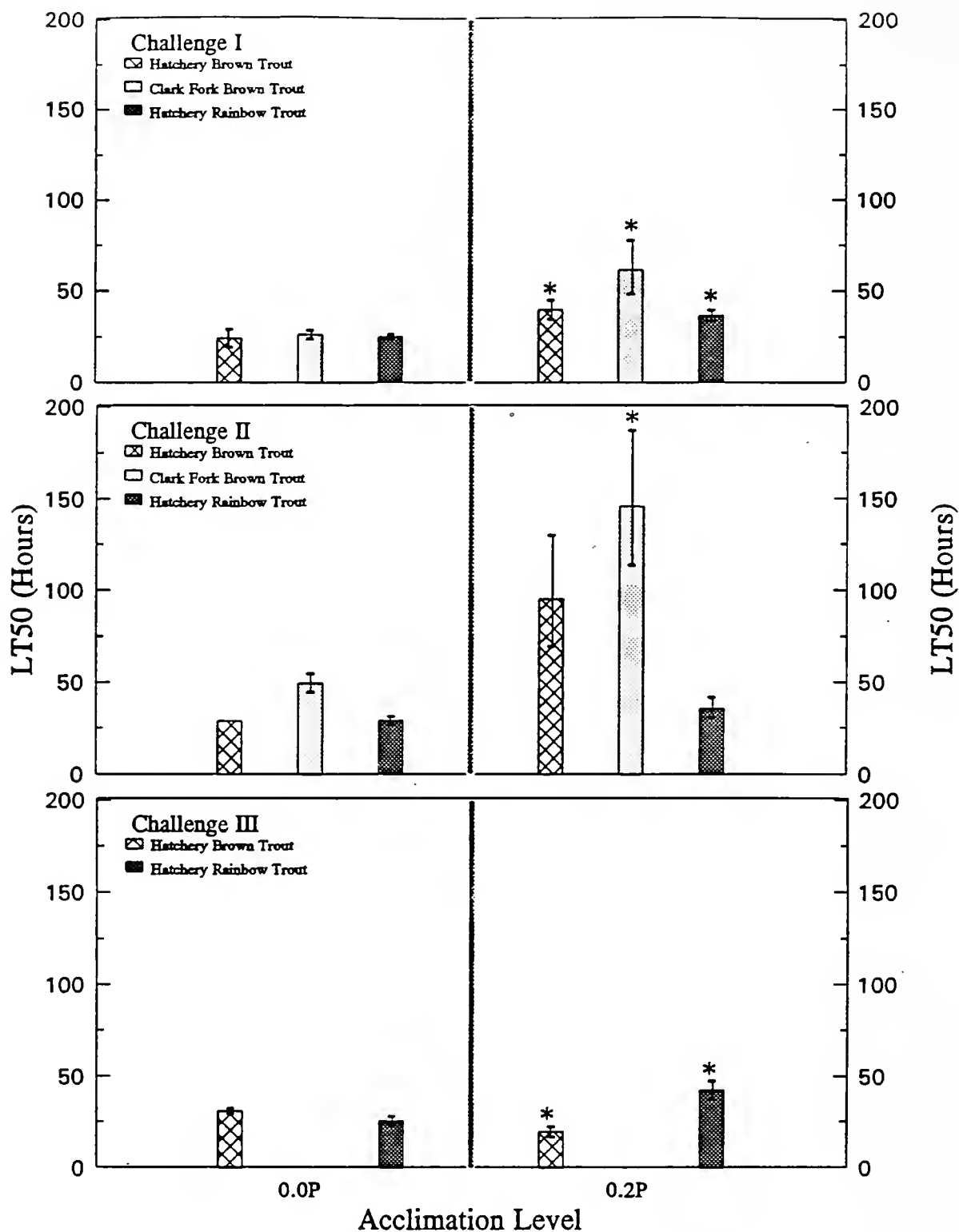Figure 5. Median lethal time (LT50) and 95% confidence limits (hours) for hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout exposed to challenge concentrations of metals following 3 and 5 weeks (Challenge I and II, respectively) of acclimation to low levels of metals (0.2P) and control (0.0P) conditions, and following 2 weeks of de-acclimation (Challenge III) in control water (0.0P) for hatchery brown and rainbow trout exposed to challenge concentrations of metals. Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. LT50 estimates and 95% confidence limits were calculated by the jackknifing method (Manly 1991); LT50 estimates with overlapping 95% confidence limits are not significantly different (α=0.05); asterisks above LT50 bars in the 0.2P acclimated group are significantly different than their paired controls. Also see Appendix Table 7.
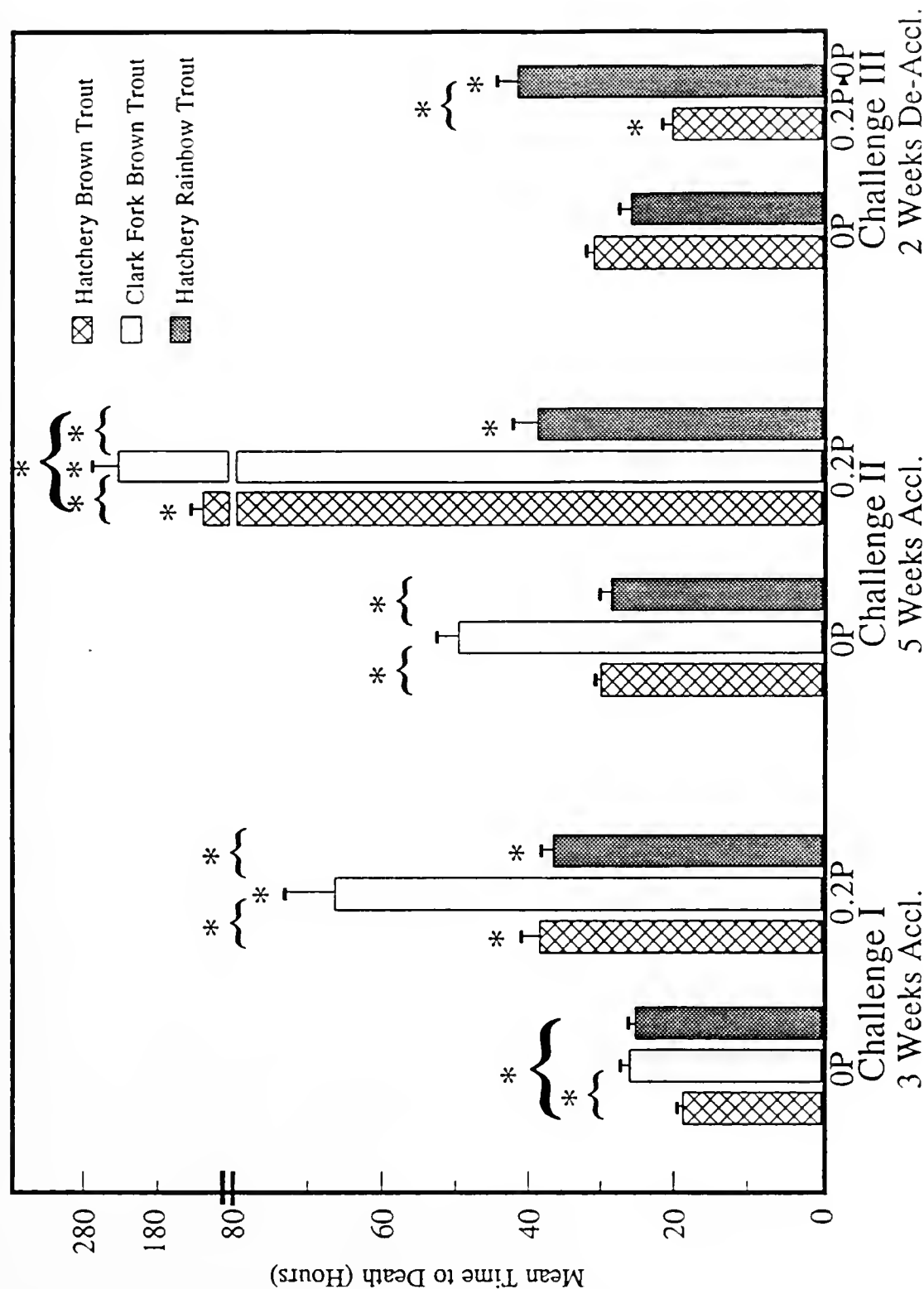
Figure 6. Mean (+1 standard error of the mean) for time to death (hours) from Challenges I, II, and III, where brown and rainbow trout were exposed to a 1P metals mixture. Challenges I and II followed 3 and 5 weeks acclimation, respectively, to low levels of metals, 0.2P, and control water, 0.0P, for hatchery brown trout, Clark Fork brown trout and hatchery rainbow trout. Challenge III followed 2 weeks de-acclimation in control water (0.0P) for hatchery brown and rainbow trout. Nominal challenge concentrations (µg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Asterisks directly above the mean values for the 0.2P or 0.2P▶0.0P acclimation groups indicate significant difference (α=0.0111 for Challenges I and II, and α=0.025 for Challenge III; Manly 1991) from the paired 0.0P acclimation group mean; an asterisk above a bracket indicates significant difference between the bracketed pair of mean values (α=0.0111 for Challenges I and II, and α=0.025 for Challenge III; Manly 1991). Also see Appendix Tables 8, 9 and 10.

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Table 1. Metal concentrations measured in the upper Clark Fork River (CFR), Montana, during storm "pulse" events and "redwater" spill events (concentrations in μg/L (ppb) total recoverable unless otherwise noted).

Date	Sample collection location	Event	Metal concentration (μg/L)			
			Zn	Cu	Pb	Cd
March 10, 1960[a]	CFR below Warm Spring Ponds	redwater	—	9,000	—	—
	CFR above East Missoula	redwater	—	3,100	—	—
May 1, 1968	CFR between Garrison and Deer Lodge	redwater	4.3	620	—	—
Nov. 20, 1968	CFR near Warm Springs	redwater	32,500	4,000	—	—
April 10, 1969	CFR at Deer Lodge	redwater	3,600	1,100	—	—
March 1, 1972	CFR near Warm Springs	redwater	500	820	—	—
May 27, 1988[b]	Mill-Willow Bypass	storm event	—	2,480	—	—
July 12, 1989[b]	Mill-Willow Bypass	storm event	14,000	13,300	30	85
	CFR below Warm Springs		800	450	10	6
	CFR at Perkins Lane		120	180	4	3
	CFR near Galen		210	370	2	3
	CFR at Deer Lodge (total recoverable)		560	330	15	3
	(dissolved)		230	120	<1	2
July 2, 1990[b]	Mill-Willow Bypass	storm event	10,300	5,800	—	—

[a] Water samples collected in association with mortality to caged fish placed instream; no dead native fish observed.
[b] Water samples collected and analyzed in association with a documented fish kill.

Table 2. Concentrations (μg/L) of metals at the mixture 96-hr LC50, the 96-hr LC50 (μg/L) reported for individual metal toxicities to rainbow trout, and individual metal contributions (%) to the Clark Fork River metals mixture at the 96-hr LC50. Toxic unit estimation and toxicity contribution estimation based on calculations as described in Parkhurst et al. (1981), and additivity index determined by calculation as described in Marking (1985).

Metal Component	A Concentration at 96-hr LC50 (μg/L) of mixture[a]	B Reference 96-hr LC50 (μg/L)[b]	C Toxic units for components (A/B)	Contribution to the mixture toxicity at the 96-hr LC50 (%) (C/Sum of Toxic Units)
Zinc	182	550[c]	0.33	16.8
Copper	127	81[d]	1.57	79.8
Lead	0.58	8,000[c]	0.00	0.0
Cadmium	2.14	32[e]	0.07	3.4

S (Sum of Toxic units) = 1.97

Additivity Index = -S + 1 = -0.97

[a] Source: Appendix Table 6

[b] 96-hr LC50 values for rainbow trout exposed to component metal at approximate hardness concentration of 100 mg/L $CaCO_3$ from indicated references.

[c] (Hale 1977, cited in USEPA 1980 and Mance 1987).

[d] (Howarth and Sprague 1978; also cited in USEPA 1984a and 1984b).

[e] (Majewski and Giles 1981, cited in USEPA 1984c and Mance 1987).

Table 3. Relative sensitivities of hatchery brown and rainbow trout as determined by the measured responses used in the various exposure conditions from the Fishery Protocols #1,2,3,4 and 5, specified under Research Plans, Section 7.4.4, in the Assessment Plan: Part I, Clark Fork River NPL Sites, Montana (Montana 1992).

Fishery Protocol	Exposure conditions	Response Endpoint	Observed Response	Relative species/ strain sensitivity
5	Acutely lethal concentrations of Clark Fork metals mixture (Range = 0 to 5P, where 1P = 230 Zn, 120 Cu, 3.2 Pb and 2.0 Cd)	96-hr LC50	Death	CF-BNT > H-BNT > H-RBT
5	same	12-hr LC50	"	CF-BNT = H-BNT = H-RBT
5	Acutely lethal concentrations of Clark Fork metals mixture (1P challenge concentration) after acclimation to 0.0P (control) metals	Mean time to death - after 3 weeks accl. - after 5 weeks accl.	" " "	H-BNT > H-RBT = CF-BNT H-BNT = H-RBT > CF-BNT
5	Acutely lethal concentrations of Clark Fork metals mixture (1P challenge concentration) after acclimation to 0.2P metals	Mean time to death - after 3 weeks accl. - after 5 weeks accl. - after 2 weeks de-accl.	" " " "	H-BNT = H-RBT > CF-BNT H-RBT > H-BNT > CF-BNT H-BNT > H-RBT
3	Acutely lethal pulse concentrations of Clark Fork metal mixture (Range = 0 to 8P) for 8 hr pulse + 96 hr post-pulse at 0.0P Pulsed exposures using fry only: - Constant hardness (100 mg/L) and constant pH (7.58) during pulse	8 + 96-hr LC50	"	H-BNT > H-RBT
	- Reduced hardness (100 to 50 mg/L) and constant pH (7.30) during pulse	8 + 96-hr LC50	"	H-RBT ≥ H-BNT
	- Reduced hardness (100 to 50 mg/L) and reduced pH (7.71 to 4.84) during pulse	8 + 96-hr LC50	"	H-RBT > > H-BNT

Table 3 (continued).

Fishery Protocol	Exposure conditions	Response Endpoint	Observed Response	Relative species/ strain sensitivity
3				
	- Reduced hardness (200 to 100 mg/L) and reduced pH (7.90 to 5.12) during pulse	8 + 96-hr LC50	Death	H-RBT > > H-BNT = CF-BNT
	- Reduced hardness (200 to 100 mg/L) and reduced pH (7.87 to 5.24) during pulse	8 + 96-hr LC50	"	H-RBT > H-BNT > CF-BNT
	- Elevated hardness (200 to 400 mg/L) and reduced pH (7.86 to 4.96) during pulse	8 + 96-hr LC50	"	H-BNT = H-RBT
	Pulsed exposures using juveniles (j) and fry (f): - Reduced hardness (200 to 100 mg/L) and reduced pH (7.87 to 4.83/5.05) during pulse	8 + 96-hr LC50	"	H-RBT j ≥ H-RBT f ≥ H-BNT f > H-BNT j
1	Chronic 88-day food/water exposure to Warm Springs diet vs. Turah Bridge diet	88-day growth	Reduced growth	H-RBT ≥ H-BNT
1,2	same	Physiology	Gut impaction	H-BNT > H-RBT
1,2	same	Physiology	Lipid peroxidation	H-BNT > H-RBT
1,2	same	Physiology	Histopathology	H-BNT > H-RBT
4	Short-term exposure to paired control and metal contaminated water in avoidance chamber (Range of metal mixture = 0.1 to 10 X)	Behavior	Avoidance	H-RBT > > H-BNT

Appendix Table 1. Mean weight and length with respective sample size (N) and ranges from the LC50 determinations for juvenile hatchery brown trout (H-BNT), Clark Fork brown trout (CF-BNT), and hatchery rainbow trout (H-RBT).

Species	N	Weight(g)	Range	Length(mm)	Range
H-BNT	240	25.03	(7.70 - 52.36)	132.5	(91.0 - 165.0)
CF-BNT	240	47.42	(19.29 - 128.25)	164.0	(124.0 - 235.0)
H-RBT	240	10.61	(3.23 - 23.74)	100.7	(71.0 - 130.0)

Appendix Table 2. Mean weight and length with respective sample size (N) and ranges from the LT50 and mean time to death determinations for juvenile hatchery brown trout (H-BNT), Clark Fork brown trout (CF-BNT), and hatchery rainbow trout (H-RBT).

	Species/ Treatment	N	Weight(g)	Range	Length(mm)	Range
	0.0P H-BNT	30	28.18	(12.14 - 44.17)	137.27	(105.00 -160.00)
CHALLENGE 1	0.0P CF-BNT	30	36.14	(12.66 - 92.01)	154.67	(110.00 - 212.00)
(3 week	0.0P H-RBT	30	23.29	(6.54 - 52.26)	130.93	(84.00 - 171.00)
acclimation)	0.2P H-BNT	30	22.08	(10.81 - 39.07)	129.73	(105.00 - 156.00)
	0.2P CF-BNT	30	25.80	(14.76 - 46.68)	141.44	(116.00 - 173.00)
	0.2P H-RBT	30	24.87	(10.18 - 36.13)	132.24	(98.00 - 151.00)
	0.0P H-BNT	30	34.17	(14.74 - 50.95)	146.93	(115.00 - 167.00)
CHALLENGE II	0.0P CF-BNT	30	70.76	(27.21 - 131.95)	190.30	(145.00 - 243.00)
(5 week	0.0P H-RBT	30	28.43	(10.49 - 51.22)	138.03	(101.00 - 170.00)
acclimation)	0.2P H-BNT	30	22.73	(7.41 - 40.17)	132.77	(97.00 - 162.00)
	0.2P CF-BNT	30	41.52	(11.58 - 110.65)	159.37	(114.00 - 230.00)
	0.2P H-RBT	30	28.56	(15.80 - 48.21)	139.07	(116.00 - 164.00)
	0.0P H-BNT	30	43.04	(21.75 - 64.10)	152.90	(126.00 - 175.00)
CHALLENGE III	0.0P H-RBT	30	43.15	(18.19 - 93.85)	149.81	(115.00 - 191.00)
(2 week	0.2P→0.0P H-BNT	30	32.37	(17.32 - 67.53)	138.27	(115.00 - 175.00)
de-acclimation)	0.2P→0.0P H-RBT	30	39.10	(13.12 - 68.80)	149.10	(108.00 - 181.00)

Appendix Table 3. Water quality conditions from the LC50 and LT50/mean time to death determinations expressed for nominal level, measured average, respective range and sample size (N) for hardness (ppm CaCO$_3$), alkalinity (ppm CaCO$_3$), and pH.

		Nominal	Avg.	Range	N
LC50	Hard.	100	101.5	(95.9 - 111.3)	42
	Alk.	80 - 110	87.3	(79.9 - 91.6)	14
	pH	7.2 - 7.8	7.66	(7.52 - 7.78)	42
0.2P EXPOSURE	Hard.	100	100.4	(95.7 - 106.1)	39
	Alk.	80 - 110	90.8	(85.8 - 95.5)	14
(Acclimation)	pH	7.2 - 7.8	7.58	(7.42 - 7.73)	39
0.0P EXPOSURE	Hard.	100	99.8	(95.7 - 106.1)	39
	Alk.	80 - 110	95.5	(85.8 - 95.5)	12
(Acclimation)	pH	7.2 - 7.8	7.58	(7.43 - 7.71)	39
CHALLENGE I	Hard.	100	100.9	(97.8 - 104.0)	18
	Alk.	80 - 110	91.0	(85.8 - 95.6)	18
	pH	7.2 - 7.8	7.56	(7.48 - 7.66)	18
CHALLENGE II	Hard.	100	100.57	(97.8 - 104.0)	25
	Alk.	80 - 110	91.1	(83.9 - 97.5)	25
	pH	7.2 - 7.8	7.60	(7.51 - 7.72)	25
CHALLENGE III	Hard.	100	99.2	(98.0 - 102.0)	8
	Alk.	80 - 110	90.3	(87.7 - 93.6)	8
	pH	7.2 - 7.8	7.60	(7.53 - 7.68)	8

Appendix Table 4. Average dissolved oxygen (ppm and % saturation) and temperature (°C) with respective range and sample size (N) from the LC50 and LT50/mean time to death determinations; values represent measurements taken once daily during the LC50 and LT50 tests and once every three days for the acclimation exposures.

	Oxygen				Temperature		
	(ppm)	(range)	(%)	(N)	(°C)	(range)	(N)
LC50	8.5	(8.2 - 8.8)	98	42	10.0	(9.8 - 10.1)	42
0.2P EXPOSURE (Acclimation)	8.3	(8.0 - 8.6)	96	39	10.0	(9.8 - 10.2)	39
0.0P EXPOSURE (Acclimation)	8.3	(7.9 - 8.6)	96	39	10.0	(9.8 - 10.5)	39
CHALLENGE I	8.2	(8.0 - 8.3)	94	18	10.0	(10.0 - 10.2)	18
CHALLENGE II	8.2	(8.0 - 8.3)	94	25	10.0	(10.0 - 10.2)	25
CHALLENGE III	8.1	(8.0 - 8.3)	93	8	10.0	(10.0 - 10.0)	8

Appendix Table 5. Measured metal concentrations in test and control waters during the LC50 and LT50/mean time to death determinations. P Units represent the notation for observed metal concentrations during pulse events in the Clark Fork River (see text), where metal concentrations (μg/L) for 1P are 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Average Zn (μg/L) concentrations (standard deviation; and sample size, N), Cu, Pb and Cd (standard deviation; and sample size, N = 2 unless otherwise indicated) determined by atomic absorption spectroscopy. Values below the method detection limit (in μg/L) of 4.9 Zn, 4.6 Cu, 1.7 Pb and 0.4 Cd are represented as "<.".

	P Unit	Zn (s.d.; N)	Cu	Pb	Cd
	5.0	1187.7 (78.4; 8)	753.0 (3.6)	16.68 (0.35)	13.05 (0.35)
LC50	2.5	590.8 (45.4; 8)	369.2 (11.3)	8.18 (0.11)	5.60 (0.49)
	1.2	297.7 (19.6; 14)	180.4 (3.9)	4.05 (__)*	3.06 (0.06)
	0.6	146.5 (9.9; 14)	92.0 (3.9)	<.	1.57 (0.04)
	0.3	68.6 (8.6; 14)	44.6 (0.0)	<.	0.70 (0.00)
	0.0	<. (__; 14)	<. (__)	<.	<.
0.2P EXPOSURE (Acclimation)	0.2P	53.0 (4.3; 39)	33.6 (4.5; 12)	<. (__; 11)	0.54 (0.09; 10)
0.0P EXPOSURE (Acclimation)	0.0P	2.8 (3.8; 39)	6.4 (5.3; 13)	<. (__; 11)	<. (__; 12)
CHALLENGE I	1.0P	260.8 (10.7; 15)	150.4 (7.3; 6)	3.51 (0.70; 6)	3.03 (0.12; 6)
CHALLENGE II	1.0P	249.1 (12.6; 28)	116.3 (9.6; 8)	3.87 (0.32; 8)	2.88 (0.10; 8)
CHALLENGE III	1.0P	287.7 (26.0; 14)	163.0 (3.8; 2)	4.50 (0.71; 2)	3.45 (0.07; 2)

* indicates sample size = 1.

Appendix Table 6. LC50 estimates (95% confidence limits in parentheses) for hatchery brown trout (H-BNT), Clark Fork brown trout (CF-BNT) and hatchery rainbow trout (H-RBT) exposed to dilutions of a mixture of Zn, Cu, Pb and Cd. LC50 values based on the measured Zn, Cu, Pb or Cd concentrations (μg/L) with the 1P unit conversion and 95% confidence intervals based on the Zn-LC50 estimate. The 1P unit conversion is as follows: 230 Zn, 120 Cu, 3.2 Pb and 2.0 Cd. LC50 calculations made using the Spearman-Karber method (Hamilton et al. 1977). For comparisons within each time period, LC50 values (in P units) shown with the same letter superscript are not significantly different, based on comparison of 95% confidence limits ($\alpha=0.05$) with overall protection of $(0.95)^3 = 0.86$. Also see Figures 2 and 3.

Time (Hours)	Species	Zn LC50	Cu LC50	Pb LC50	Cd LC50	P Units LC50
12	H-BNT	742 (683, 806)	465 (427, 507)	10.32 (9.48, 11.24)	7.53 (6.90, 8.22)	3.23 (2.97, 3.51) [a]
	CF-BNT	626 (557, 703)	390 (346, 440)	8.67 (7.70, 9.77)	6.30 (5.57, 7.12)	2.72 (2.42, 3.06) [a]
	H-RBT	757 (691, 828)	475 (433, 521)	10.53 (9.60, 11.55)	7.68 (6.98, 8.44)	3.39 (3.01, 3.60) [a]
24	H-BNT	405 (379, 433)	249 (233, 267)	5.08 (4.31, 5.97)	4.02 (3.77, 4.28)	1.76 (1.65, 1.88) [ab]
	CF-BNT	346 (309, 388)	213 (190, 239)	3.21 (2.41, 4.27)	3.49 (3.14, 3.88)	1.50 (1.34, 1.69) [a]
	H-RBT	431 (408, 454)	267 (255, 281)	5.96 (5.68, 6.26)	4.29 (4.08, 4.51)	1.87 (1.78, 1.98) [b]
48	H-BNT	164 (146, 185)	103 (92, 115)	0.14 (0.06, 0.31)	1.72 (1.53, 1.93)	0.71 (0.64, 0.80) [a]
	CF-BNT	170 (151, 192)	106 (95, 119)	0.17 (0.08, 0.37)	1.78 (1.58, 2.00)	0.74 (0.66, 0.83) [a]
	H-RBT	240 (215, 267)	148 (133, 165)	1.06 (0.67, 1.65)	2.48 (2.24, 2.74)	1.04 (0.94, 1.16) [b]
72	H-BNT	139 (124, 157)	88 (79, 98)	0.05 (0.02, 0.11)	1.46 (1.30, 1.64)	0.61 (0.54, 0.68) [a]
	CF-BNT	137 (122, 154)	86 (77, 96)	0.04 (0.02, 0.09)	1.43 (1.28, 1.61)	0.60 (0.53, 0.67) [a]
	H-RBT	219 (197, 244)	136 (122, 151)	0.70 (0.40, 1.22)	2.28 (2.05, 2.52)	0.95 (0.85, 1.06) [b]
96	H-BNT	132 (118, 148)	83 (75, 93)	0.03 (0.01, 0.07)	1.38 (1.23, 1.55)	0.57 (0.51, 0.64) [a]
	CF-BNT	97 (85, 112)	78 (70, 87)	0.02 (0.01, 0.04)	1.29 (1.15, 1.44)	0.42 (0.37, 0.49) [b]
	H-RBT	182 (147, 226)	127 (112, 144)	0.58 (0.27, 1.24)	2.14 (1.89, 2.42)	0.79 (0.64, 0.98) [c]

Appendix Table 7. LT50 estimates (hours) and 95% confidence limits for hatchery brown trout (H-BNT), Clark Fork brown trout (CF-BNT) and hatchery rainbow trout (H-BNT) exposed to challenge concentrations of metals following 3 and 5 weeks (Challenges I and II, respectively) of acclimation to low levels of metals (0.2P) and control (0.0P) conditions and following 2 weeks of de-acclimation in control water (Challenge III; see text). Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Complete mortality occurred in all exposures unless indicated otherwise. LT50 estimates and 95% confidence limits were calculated by the jackknifing method (Manly 1991); LT50 estimates shown with the same letter superscript within a challenge group are not significantly different based on comparison of 95% confidence limits (α=0.05). Also see Figures 4 and 5.

Species	Acclimation condition	LT50 (hrs) (95% c.l.)
Challenge I (3 weeks acclimation)		
H-BNT	0.0P	24.46 (19.54, 29.39) ▼ [a]
CF-BNT	0.0P	26.39 (24.03, 28.75) [a]
H-RBT	0.0P	25.13 (23.69, 26.65) [a]
H-BNT	0.2P	39.85 (34.64, 45.05) [b]
CF-BNT	0.2P	61.37 (48.47, 77.71) [c]
H-RBT	0.2P	36.78 (33.91, 39.65) [b]
Challenge II (5 weeks acclimation)		
H-BNT	0.0P	29.10 (no c.l.)#
CF-BNT	0.0P	49.54 (44.69, 54.91) [a]
H-RBT	0.0P	29.39 (27.12, 31.67) [b]
H-BNT	0.2P	95.65 (70.30, 130.13) [c]
CF-BNT	0.2P	146.19 (113.96, 187.55)* [c]
H-RBT	0.2P	35.77 (30.53, 41.91) [b]
Challenge III (2 weeks de-acclimation)		
H-BNT	0.0P	31.29 (29.67, 32.91) [a]
H-RBT	0.0P	25.80 (23.33, 28.27) [b]
H-BNT	0.2P→0.0P	19.91 (17.14, 22.68) [c]
H-RBT	0.2P→0.0P	42.67 (37.57, 47.76) [d]

▼ failed goodness of fit test for normal distribution.

estimate made by graphical interpolation because jacknifed LT50 calculations were not possible due to low variance.

* 67% mortality @ 480 hrs.

Appendix Table 8a. Mean, standard deviation, and standard error of the mean for time to death (hours) from Challenge I (3 weeks acclimation) for each combination of the two factors where the first factor, Species, contains three levels (hatchery brown trout, H-BNT; Clark Fork brown trout, CF-BNT; and hatchery rainbow trout, H-RBT) and the second factor, Acclimation, contains two levels (0.0P = control water acclimation, and 0.2P = low levels of metals acclimation). Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Mean values shown with the same letter superscript are not significantly different based on t-test results shown below in Appendix Table 8c. Also see Figure 6.

Species	Acclimation condition	N	Mean (hrs)	S.D.	S.E.M.
H-BNT	0.0P	30	18.733 [a]	4.193	0.776
CF-BNT	0.0P	30	26.000 [b]	7.061	1.289
H-RBT	0.0P	30	25.200 [c]	5.492	1.003
H-BNT	0.2P	30	38.400 [d]	14.036	2.563
CF-BNT	0.2P	30	66.400 [e]	36.401	6.646
H-RBT	0.2P	30	36.600 [d]	9.133	1.667

Appendix Table 8b. Two factor Analysis of Variance (ANOVA) results for Challenge I where the first factor, Species, contains three levels (hatchery brown trout, H-BNT; Clark Fork brown trout, CF-BNT; and hatchery rainbow trout, H-RBT) and the second factor, Acclimation, contains two levels (0.0P = control water acclimation, and 0.2P = low levels of metals acclimation). P-values below $\alpha=0.10$ were judged to be significant (*).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	11009.37778	5504.68889	19.39	0.0001 *
ACCLIM	1	25537.42222	25537.42222	89.97	0.0001 *
SPECIES*ACCLIM	2	6696.04444	3348.02222	11.80	0.0001 *
ERROR	174	49388.26667	283.84061		
TOTAL	179	92631.11111			

Appendix Table 8c. *A priori* t-test results for Challenge I for various combinations of the two factors, Species (hatchery brown trout, H-BNT, Clark Fork brown trout, CF-BNT, hatchery rainbow trout, H-RBT) and Acclimation condition (0.0P = control water acclimation, and 0.2P = low levels of metals acclimation). P-values for tests of equality of variances (Pr > F') were judged to be significant at the $\alpha=0.01$ level, and degrees of freedom were adjusted by Satterthwaite's correction when hypothesis of equal variance was rejected. P-values for tests of equal mean time to death were judged to be significant (*) at the conservative Bonferonni adjusted value (Manly 1991) of $\alpha=0.10/9=0.0111$. Also see Figure 6.

Comparison	F'	Pr>F'	| T |	DF	Pr > | T |
H-BNT 0.0P vs H-BNT 0.2P	11.21	0.0001	7.3534	34.1	0.0001 *
CF-BNT 0.0P vs CF-BNT 0.2P	26.57	0.0001	5.9678	31.2	0.0001 *
H-RBT 0.0P vs H-RBT 0.2P	2.77	0.0078	5.8587	47.5	0.0001 *
CF-BNT 0.0P vs H-BNT 0.0P	2.84	0.0065	4.8465	47.2	0.0001 *
CF-BNT 0.0P vs H-RBT 0.0P	1.65	0.1820	0.4898	58.0	0.6261
H-BNT 0.0P vs H-RBT 0.0P	1.72	0.15220	5.1259	58.0	0.0001 *
CF-BNT 0.2P vs H-BNT 0.2P	6.73	0.0001	3.9311	37.4	0.0004 *
CF-BNT 0.2P vs H-RBT 0.2P	15.88	0.0001	4.3492	32.6	0.0001 *
H-BNT 0.2P vs H-RBT 0.2P	2.36	0.0238	0.5887	58.0	0.5583

Appendix Table 9a. Mean, standard deviation, and standard error of the mean for time to death (hours) from Challenge 11 (5 weeks acclimation) for each combination of the two factors where the first factor, Species, contains three levels (hatchery brown trout, H-BNT; Clark Fork brown trout, CF-BNT; and hatchery rainbow trout, H-RBT) and the second factor, Acclimation, contains two levels (0.0P = control water acclimation, and 0.2P = low levels of metals acclimation). Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Mean values shown with the same letter superscript are not significantly different based on t-test results shown below in Appendix Table 9c. Also see Figure 6.

Species	Acclimation condition	N	Mean (hrs)	S.D.	S.E.M.
H-BNT	0.0P	30	30.000 [a]	4.386	0.801
CF-BNT	0.0P	30	49.600 [b]	16.198	2.957
H-RBT	0.0P	30	28.600 [a]	8.621	1.574
H-BNT	0.2P	30	120.067 [c]	102.777	18.764
CF-BNT	0.2P	30	244.600 [d]	179.763	32.820
H-RBT	0.2P	30	38.800 [e]	18.340	3.348

Appendix Table 9b. Two factor Analysis of Variance (ANOVA) results for Challenge II where the first factor, Species, contains three levels (hatchery brown trout, H-BNT; Clark Fork brown trout, CF-BNT; and hatchery rainbow trout, H-RBT) and the second factor, Acclimation, contains two levels (0.0P = control water acclimation, and 0.2P = low levels of metals acclimation). P-values below $\alpha=0.10$ were judged to be significant (*).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	395232.1778	197616.0889	27.21	0.0001 *
ACCLIM	1	435912.0222	435912.0222	60.03	0.0001 *
SPECIES*ACCLIM	2	257703.6444	128851.8222	17.74	0.0001 *
ERROR	174	1263534.267	7261.691		
TOTAL	179	2352382.111			

Appendix Table 9c. *A priori* t-test results for Challenge II for various combinations of the two factors, Species (hatchery brown trout, H-BNT, Clark Fork brown trout, CF-BNT, hatchery rainbow trout, H-RBT) and Acclimation condition (0.0P = control water acclimation, and 0.2P = low levels of metals acclimation). P-values for tests of equality of variances (Pr>F') were judged to be significant at the $\alpha=0.01$ level, and degrees of freedom were adjusted by Satterthwaite's correction when hypothesis of equal variance was rejected. P-values for tests of equal mean time to death were judged to be significant (*) at the conservative Bonferonni adjusted value (Manly 1991) of $\alpha=0.10/9=0.0111$. Also see Figure 6.

Comparison	F'	Pr>F'	| T |	DF	Pr > | T |
H-BNT 0.0P vs H-BNT 0.2P	548.97	0.0001	4.7955	29.1	0.0001 *
CF-BNT 0.0P vs CF-BNT 0.2P	123.16	0.0001	5.9175	29.5	0.0001 *
H-RBT 0.0P vs H-RBT 0.2P	4.53	0.0001	2.7568	41.2	0.0087 *
CF-BNT 0.0P vs H-BNT 0.0P	13.64	0.0001	6.3970	33.2	0.0001 *
CF-BNT 0.0P vs H-RBT 0.0P	3.53	0.0011	6.2684	44.2	0.0001 *
H-BNT 0.0P vs H-RBT 0.0P	3.86	0.0005	0.7928	43.1	0.4323
CF-BNT 0.2P vs H-BNT 0.2P	3.06	0.0036	3.2940	46.1	0.0019 *
CF-BNT 0.2P vs H-RBT 0.2P	96.07	0.0001	6.2382	29.6	0.0001 *
H-BNT 0.2P vs H-RBT 0.2P	31.40	0.0001	4.2636	30.8	0.0002 *

Appendix Table 10a. Mean, standard deviation, and standard error of the mean for time to death (hours) from Challenge III (2 weeks de-acclimation) for each combination of the two factors where the first factor, Species, contains two levels (hatchery brown trout, H-BNT; and hatchery rainbow trout, H-RBT) and the second factor, Acclimation, contains two levels (0.0P = control water acclimation, and and 0.2P→0.0P = 2 weeks de-acclimation in 0.0P control water). Nominal challenge concentrations (μg/L) for the 1P challenge exposure were 230 Zn, 120 Cu, 3.2 Pb, and 2.0 Cd. Mean values shown with the same letter superscript are not significantly different based on t-test results shown below in Appendix Table 10c. Also see Figure 6.

Species	Acclimation condition	N	Mean (hrs)	S.D.	S.E.M.
H-BNT	0.0P	30	30.867 [a]	7.219	1.318
H-RBT	0.0P	30	25.867 [a]	8.846	1.615
H-BNT	0.2P→0.0P	30	20.200 [b]	7.568	1.382
H-RBT	0.2P→0.0P	30	41.600 [c]	15.251	2.784

Appendix Table 10b. Two factor Analysis of Variance (ANOVA) results for Challenge III where the first factor, Species, contains two levels (hatchery brown trout, H-BNT; and hatchery rainbow trout, H-RBT) and the second factor, Acclimation, contains two levels (0.0P = control water, and 0.2P→0.0P = 2 weeks de-acclimation in 0.0P control water). P-values below $\alpha = 0.10$ were judged to be significant (*).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	1	3967.500000	3967.500000	32.43	0.0001 *
ACCLIM	1	1020.833333	1020.833333	8.34	0.0046 *
SPECIES*ACCLIM	1	2940.300000	2940.300000	24.03	0.0001 *
ERROR	116	14193.333333	122.356322		
TOTAL	119	22121.966667			

Table 10c. *A priori* t-test results for Challenge III for various combinations of the two factors, Species (hatchery brown trout, H-BNT, hatchery rainbow trout, H-RBT) and Acclimation condition (0.0P = control water acclimation, and 0.2P→0.0P = 2 weeks de-acclimation in 0.0P control water). P-values for tests of equality of variances (Pr > F') were judged to be significant at the $\alpha = 0.01$ level, and degrees of freedom were adjusted by Satterthwaite's correction when hypothesis of equal variance was rejected. P-values for tests of equal mean time to death were judged to be significant (*) at the conservative Bonferonni adjusted value (Manly 1991) of $\alpha = 0.10/4 = 0.025$. Also see Figure 6.

Comparison	F'	Pr > F'	| T |	DF	Pr > | T |
H-BNT 0.0P vs H-BNT 0.2P→0.0P	1.10	0.8015	5.5860	58.0	0.0001 *
H-RBT 0.0P vs H-RBT 0.2P→0.0P	2.97	0.0045	4.8877	46.5	0.0001 *
H-BNT 0.0P vs H-RBT 0.0P	1.50	0.2795	2.3984	58.0	0.2795
H-BNT 0.2P→0.0P vs H-RBT 0.2P→0.0P	4.06	0.0003	6.8846	42.5	0.0001 *

AQUATIC RESOURCES INJURY REPORT

APPENDIX D

Determine the Extent to Which Rainbow Trout and Brown Trout Avoid or Prefer Water Quality Characteristic of the Clark Fork River

Prepared by:

Dan Woodward, U.S. Fish and Wildlife Service
Harold Bergman, University of Wyoming

Determine the Extent to Which Rainbow Trout and Brown Trout Avoid
or Prefer Water Quality Characteristic of the Clark Fork River

D.F. Woodward and H.L. Bergman

INTRODUCTION

In the Clark Fork River, copper (Cu) and zinc (Zn) exist in
surface water at concentrations that frequently exceed water
quality criteria; and cadmium (Cd) and lead (Pb) occasionally
exceed water quality criteria (USEPA 1987, USGS 1989, Lambing
1991). While these criteria are formulated to protect survival,
growth, and reproduction of aquatic life, there is evidence that
behavioral avoidance of Cd, Cu, Pb, and Zn concentrations in
surface water may be an additional cause of reduced fish
populations in the river (Sprague 1968, Folmar 1976, Giattina et
al. 1982, Giattina and Garton 1983, McNicol and Scherer 1990).

Studies in both the laboratory and field have documented the
avoidance of harmful environmental conditions by fish (Beitinger
and Freeman 1983, Rice 1973, Gunn and Noakes 1986, Hartwell et
al. 1987, Hartwell et al. 1989). Ability of fish to sense and
avoid unfavorable conditions has been used to establish threshold
concentrations for undesirable substances like carbon dioxide,
ammonia, copper, and lead. Salmonids are particularly sensitive
to copper, avoiding solutions as low as 0.1 $\mu g/l$ (Folmar 1976).
Introduction of copper and zinc into a salmon spawning tributary
resulted in repulsion of ascending salmon as compared to the same
tributary prior to mine drainage release (Saunders and Sprague
1967). Furthermore, the Department of Interior regulations for

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natural resource damage assessment, states that behavioral avoidance meets the acceptance criteria for demonstrating injury to a biological resource (43 CFR 11.62 (s) (4) (iii) (B)).

High concentrations of copper may disable or destroy certain sensory perception and cause decreased avoidance (Gardner and LaRoche 1973), allowing salmonids to be exposed to the contamination and the possibility of death. Some laboratory studies have suggested that in addition to avoiding low concentrations of metals, fish show preference to high concentrations of copper (i.e., 390 μg/l) (Giattina et al. 1982).

There were four objectives to this protocol. 1) Determine the avoidance/preference response of rainbow trout and brown trout to water and metals concentrations simulating the Clark Fork River. 2) Determine if decreased pH of storm events influences the avoidance/preference response of rainbow trout and brown trout. 3) Determine if rainbow trout acclimated to Clark Fork River conditions will be attracted to waters simulating tributaries with low metals. 4) Determine if rainbow trout in simulated soft or hard water of tributaries with low metals concentrations would be attracted to Clark Fork River conditions.

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METHODS

Test Fish. Rainbow trout and brown trout used in testing
were obtained as eggs from the Ennis National Fish Hatchery in
Montana and the Saratoga National Fish Hatchery in Wyoming. Both
species were cultured at the National Fisheries Contaminant
Research Center (NFCRC) Field Station in Jackson, Wyoming. Test
fish were in good condition and free of obvious disease, injury,
or distress. Acclimation to changes in temperature and hardness
was at least 72-h. Changes in temperature did not exceed 1°C per
day, and changes in hardness did not exceed a 25% per day.

All fish used in testing were handled to minimize stress.
Fish collected for testing from the acclimation tanks were
handled and transported quickly to the avoidance chamber. The
fish were deprived of food for a minimum of 12 hours prior to
testing, but were not deprived for more than 24 hours. A single
fish from 55 to 85 millimeters long was used for each test.

Reference and Test water. Specific reference and test water
characteristics are defined later under each task. In general
reference and test waters used in this experiment simulated one
of the following conditions: upper Clark Fork River without
metals, upper Clark Fork River with metals, a soft water
tributary without metals, or a hard water tributary without
metals. The pH for each water was 8.0 unless indicated
otherwise. Alkalinity and hardness were reported in mg/L as
$CaCO_3$, conductivity was reported as uM/cm.

Simulated Clark Fork River water had a hardness of 100,

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alkalinity of 100, and conductivity of 200. Simulated Clark Fork
River water had metals present in an aqueous mixture of 1.1 ug/L
Cd ($CdCl_2$), 12 ug/L Cu ($CuCl_2$), 3.2 ug/L Pb ($PbCl_2$), and 50 ug/L
Zn ($ZnCl_2$) which was the 1X treatment. The 1X metals are
representative of ambient concentrations measured in the Clark
Fork River (Lambing 1991), and also represents the chronic
ambient water quality criteria at a water hardness of 100 mg/L
(except for Zn which has a criterion of 110 ug/L). There were
six additional metals concentrations tested: 0X (no metals),
0.1X, 0.5X, 2X, 4X, and 10X. The simulated soft water tributary
had a hardness of 50, alkalinity of 50, and conductivity of 140.
The simulated hard water tributary had a hardness of 200,
alkalinity of 160, and conductivity of 510.

Each water was reconstituted by mixing appropriate
quantities of deionized water with well water into storage tanks.
Metals were obtained as individual reagent grade salts from VWR
Scientific and measured individually into a common stock
container. Where adjustments in pH were needed H_2SO_4 was used.

Reference water was used for acclimation and as an
alternative choice for the fish to the test water. Test water
varied metals concentration, pH, and hardness. Each combination
of reference and test water was a treatment. A task was a series
of treatments evaluating the avoidance response of fish to a
variable in the test water.

Chemical analyses of water. All test and reference waters
were analyzed for hardness, alkalinity, pH, conductivity, and

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metals during each day of testing. The values for these parameters were within 10% of the target values. Variations in water hardness were made by the addition of reagent grade $CaSO_4$, $MgSO_4$, $NaHCO_3$, and KCl. Water temperature was maintained at $12\pm1°C$ during acclimation and testing.

Filtered water samples were taken from test water each day of testing and from reference water four times during each task. Water samples were filtered using a Nalgene® 300 filter holder, transferred to a pre-cleaned 125 ml I-Chem® polyethylene bottle, and preserved by addition of 1 ml Ultrex-II® nitric acid. Determination of dissolved Cd, Cu, Pb, and Zn in these samples was done by graphite furnace atomic absorption spectrophotometry at Red Buttes chemistry lab. Instrument detection limits for Cd, Cu, Pb, and Zn were 0.4, 1.2, 1.7, and 4.9 ug/L.

When pH was a variable in the test water, pH was measured in the reference and test water two times during each avoidance test with an Orion^R Model EA 940.

Test apparatus. Water of the desired quality and temperature was mixed and maintained in two 800 L polypropylene reference water tanks (1A and 2A) and two 400 L polypropylene test water tanks (3A and 4A, Figure 1). Water from each A-series tank was pumped to a separate 94 L glass tank (1B, 2B, 3B, and 4B) which was connected to a 54 L glass head tank (1C, 2C, 3C, and 4C). To maintain a constant head pressure, water was pumped in excess from each B-series tank to its respective head tank (C-series) which overflows through a standpipe and returns to the

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B-series tanks. Reference water head tank 1C was paired with test water head tank 3C, to supply water to one avoidance chamber. Both ends of the chamber were identical, and reference or test water could be introduced into either end by glass valves. A second identical avoidance chamber received water from head tanks 2C and 4C. Flow rate from each head tank was 1,275±100 ml per min.

A glass mixing box was placed between each head tank and each end of the avoidance chamber. The mixing box had a series of baffles to aid in the mixing of water, metals solution, and acid. Water entered the mixing box from the head tank and exited to the avoidance chamber. When test water was needed, metals solution or acid (H_2SO_4) was metered into the incoming water with an automatic pipet (MicromedicR). Metals solution was injected at 2 ml/min and acid was injected at 2-8 ml/min. All mixing boxes were rinsed with a pH 2.0, HCl solution followed by three water rinses between each test to remove residual metals.

The avoidance chamber was a plexiglass cylinder (11 cm diameter by 92 cm) similar to the counter-current design used by Sprague (1968) (Figure 2). Water enters 5 cm from the ends of the chamber, flows towards the center, and exits through six drain holes. Plastic screens were placed 11 cm from each end of the chamber to create a 70 cm observation area. The bottom of the chamber was covered with contact paper to provide an even background. Both chambers were surrounded with black plastic to minimize external disturbance. The top of the enclosed area was

open to allow adequate lighting and use of a video camera for viewing and taping fish position from a remote location. Small low intensity lights were used to indicate reference and test end of chamber.

Fluorescein dye testing on the chambers showed a defined gradient was established 5 min after introduction of the dye. The gradient remained within 5 cm of the center of the drain holes at all times and quickly re-established following fish movement from one end to the other. In the dye tests, the dye color cleared the test end in the 20 minute rinse period.

Temperature control in each A-series tank was achieved by circulating 2 to 4°C water through polypropylene tubing coiled inside the tank. Water inside the tubing was cooled by Remcor water chillers in an external tank. Submersible pumps in the external tank circulated chilled water through the tubing on demand (12 ± 1°C) by Goldline[R] [SP33] temperature sensors inside the A-series tanks. Temperature stratification within each A-series tank was eliminated by recirculating the water with an external pump. Water of the correct temperature was transferred to the B-series tanks held in a water bath maintained at 12 ± 1°C by Remcor water chillers. Because of the rapid recycling in the C-series tanks and the flow through the avoidance chamber, no additional cooling was necessary to achieve the 12 ± 1°C in the avoidance chamber. Temperature differential between test and reference end of avoidance chamber was no more than 0.4°C.

Test procedure. One avoidance test included three phases: a

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20 min rinse phase, followed by a 20 min acclimation, and ending in a 30 min test. The rinse phase had reference water flowing into both ends of the chamber with no fish present. The acclimation phase was initiated by placing one fish in a predetermined end of the chamber. The video recorder was activated and the on-screen timer started. In the test phase, the Micromedic^R was activated and test water replaced the reference water in a randomly determined end of the chamber. The first 10 min of the test phase was considered a latency period where the contaminant gradient was established. Actual avoidance observations were made during the final 20 min of the test phase. After testing, the fish was removed from the chamber, and length and weight were measured.

For an avoidance test to be acceptable, fish had to cross the center of the avoidance chamber a minimum of ten times during the 30 min test period. This criteria was used to assure fish were experiencing the test and reference side of the avoidance chamber. A test was considered invalid if the temperature differential between the two ends of the chamber was greater than 0.4°C.

Each avoidance test was videotaped from the beginning of the acclimation period to the end of the test period. During the last 20 min of the test period, the following observations were recorded: time spent in the test water end of the chamber, number of trips into the test water end of the chamber, and mean trip time. One trip was defined as entrance into and exit out of the

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test end of the chamber.

Four avoidance tests were performed on a treatment in one avoidance chamber on each day of testing and was defined as one trial. Using two avoidance chambers we completed two trials per day. We performed six replicate trials per treatment.

Task 1: Avoidance of metals. Determine if rainbow trout and brown trout have a preference or avoidance to metals concentrations characteristic of the Clark Fork River under both normal and extreme conditions. Seven treatments were evaluated for each species. Reference water was simulated Clark Fork River water with 0X metals; test water was simulated Clark Fork River water with 0X, 0.1X, 0.5X, 1X, 2X, 4X, or 10X metals concentrations with the 0X as the control.

Task 2A: Avoidance of acidity. Determine if short-term increases in acidity associated with storm events influence the avoidance response of rainbow trout and brown trout. Four treatments were evaluated for each species. Reference water was simulated Clark Fork River water with 0X metals; and test water was the same water at four pH's: 5.0, 6.0, 7.0, and 8.0, with 8.0 as the control.

Task 2B: Avoidance of acidity and metals. Determine if short-term increases in acidity associated with storm events influence the avoidance response of rainbow trout and brown trout to water with a 1X metals concentration. Four treatments were evaluated for each species. Reference water was Clark Fork River water with 0X metals; and test water was Clark Fork River water

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with 1X metals and a pH of 5.0, 6.0, 7.0, and 8.0, with 8.0 as the control.

Task 3:   Acclimation to Clark Fork River water -- Influence on the avoidance/preference response. Determine if acclimation to simulated Clark Fork River water with 1X metals has an effect on the avoidance response of rainbow trout. Three treatments were evaluated. Reference water for this task was simulated Clark Fork River water at 1X, to which fish were acclimated 30-days before testing. Test water was simulated Clark Fork River water at 0X, 1X, and 4X; 1X was the control.

Task 4:   Acclimation to hard and soft tributary water -- Influence on avoidance/preference response. Determine if rainbow trout in tributary waters of differing water hardness avoid water characteristics of the Clark Fork River. Reference waters for this test were two simulated tributaries: soft water tributary and hard water tributary. Fish were acclimated to the reference water for 4-days before testing. Test waters were simulated Clark Fork River water with 1X and 0X metals concentraions. The 0X metals concentration was the control within a tributary, but the design allowed comparison between tributaries.

Interlaboratory validation. This task was a duplication of Task 1, but performed at the National Fisheries Contaminant Research Center, Columbia, Missouri. The objective of this task was to test the reproducibility of the avoidance response observed in Task 1 when tested at a different time and place. Methods were similar to those in Task 1, Jackson except as

indicated. Three avoidance chambers were used simultaneously in a type V balanced incomplete block design (Cochran and Cox 1957) for brown trout, and there were two fish per test. With rainbow trout only 0X, 0.5X, and 1X test waters were evaluated and three avoidance chambers were utilized in a randomized complete block design.

Data analysis and statistics. Testing restrictions did not allow all treatments to be tested simultaneously, so we used a balanced incomplete block design to eliminate time as an experimental variable (Cochran and Cox 1957; personal communication, Mark Ellerseick, Ph.D., Mathematical Sciences Department, University of Missouri, Columbia, Missouri). For each day, the two treatments to be tested were assigned on a rotating schedule from all treatments until six replicate trials were performed per treatment (Figure 3). Therefore each task evaluated either three, four, or seven treatments over a 9, 12, or 21 day period, respectively. Each treatment was compared to every other treatment equally often and with the same precision during a task.

Each treatment tested within a task was randomly assigned to test day and avoidance chamber. Test end of the avoidance chamber was determined at random for the first observation, thereafter the test end was alternated for each succeeding observation. Placement of fish into the avoidance chamber was alternated between ends.

Each trial was the experimental unit, and each trial was

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made up of four avoidance tests. The response for a trial was represented by the mean of the observations measured from the four avoidance tests. A natural log transformation was performed on the trial mean for each observation, and analysis of variance and Tukey's least significant difference tests were performed to compare differences between treatment means ($P \leq 0.05$).

RESULTS

General. The amount of time in the test water end of the avoidance chamber was a function of the number of trips into the test water and the length of time for each trip. If there was no preference or avoidance, the fish would be expected to spend equal time in the test water (600 sec, 50%) and the reference water (600 sec, 50%). The response of brown trout to metals in water (Task 1) can be used to illustrate the measurement of the avoidance/preference response throughout this experiment (Figure 4). In general, avoidance for brown trout was significant when total time in the test water was reduced below 445 sec, or 37% of the total time. Conversely, preference would have occurred when total time in the test water was greater than 776 sec, or 65% of the total time. For rainbow trout, avoidance was significant at less than 425 sec (35%), and preference was significant at greater than 892 sec (74%) (Figure 5). Actual significance was always determined by the statistical procedure described and took into account the variation and sample size within a task and species.

Task 1: Avoidance of metals. Except for Pb, metals measured in test water were within 10% of the nominal concentration (Table 1). Measured Pb concentrations were 30-40% higher than the nominal concentration in the rainbow trout 1X and 2X test waters and the brown trout 2X test waters. In the lowest test water, 0.1X nominal metals concentrations (ug/L) were Cd, 0.11; Cu, 1.2; Pb, 0.32; and Zn, 5.0. In the 0.1X water, both Cd

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and Pb were below the measurable detection limits; in the 0.5X
test water, Cd and Pb were at their detection limits.

Brown trout significantly avoided simulated Clark Fork River
water when metals concentrations were 0.5X or greater (Table 2,
Figure 4). Total time in test water dropped from 50% for 0X test
water to less than 20% for 0.5X, 1X, 2X, and 4X test waters. The
greatest avoidance was observed at 2X and 1X when brown trout
spent 8 and 13% of their time in the test water. Compared to 1X,
2X, and 4X, there was less avoidance of 10X test water by brown
trout (28% time in test water).

Rainbow trout significantly avoided simulated Clark Fork
River water at all metal concentrations, 0.1X to 10X (Table 3,
Figure 5). Total time in test water dropped from 52% for 0X test
water to less than 8% for the 0.1X test water, and was less than
2% for 0.5X, 1X, 2X, 4X, and 10X test waters. Compared to 1X,
2X, and 4X, there was no reduced avoidance at 10X with rainbow
trout as was observed with brown trout.

Rainbow trout had a more sensitive avoidance response to
metals than brown trout (Tables 2 and 3). Rainbow trout avoided
a lower concentration of test water (0.1X) and avoided each test
water to a greater extent than brown trout. Comparing the number
of trips and individual trip time between species and the same
test water, generally rainbow trout took one-half as many trips,
and trip time was one-third as long as brown trout.

Task 2: Avoidance of acidity and metals. During avoidance
testing of acidity (Task 2A and 2B), pHs of 8.0, 7.0, and 6.0

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were maintained at ±0.25 pH units; pH of 5.0 was maintained at ±0.5 pH units. The number of measurements for each pH treatment was 48. The mean measured metal concentration was generally within 10% of the nominal value for each test water in Task 2B · (Table 4).

Brown trout significantly avoided simulated Clark Fork River water (0X) at pHs of 7.0, 6.0, and 5.0 (Table 5). Total time in test water dropped from 48% for pH 8.0, to less than 20% for pH of 7.0, to less than 10% for pH 6.0 and 5.0. When 1X metals were presented in combination with pH 8.0, brown trout spent only 13% of their time in the test water (Table 6) which is similar to the results observed in Task 1 for 1X test water. Total time in test water of pH 7.0, 6.0, and 5.0 and 1X metals was less than 10%, but not significantly below the response observed in pH 8.0 1X.

Rainbow trout significantly avoided simulated Clark Fork River water (0X) at pH's of 7.0, 6.0, and 5.0 (Table 7). Each successive drop in pH significantly increased the avoidance response. Percentage of time spent in test water was 57% for pH 8.0, 11% for pH 7.0, 3.8% for pH 6.0, and 2.3% for pH 5.0. When 1X metals were presented in combination with pH 8.0, rainbow trout spent only 5.2% of their time in the test water (Table 8) which is similar to the results observed in Task 1 for 1X test water. Total time in test water of pH 6.0 1X and pH 5.0 1X was less than 3%, and significantly below the pH 8.0 1X response.

As in Task 1 the avoidance response of rainbow trout to reduced pH and elevated metals was more sensitive than brown

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trout. For both pH only tests and pH plus 1X metals tests, rainbow trout spent less time in the test water than brown trout.

Task 3: Acclimation to Clark Fork River water -- Influence on the avoidance/preference response. During the 30 d acclimation to Clark Fork River water with 1X metals, measured concentrations were within 10% of the nominal values except for Pb which was 30% low (Table 9). The reference water was 1X in this task and the 1X reference water and 1X test water were almost identical in metals concentrations. Metal concentrations in the 0X and 4X test waters were close to the nominal concentrations to be expected from the ratios.

Even though rainbow trout were acclimated to Clark Fork River water with 1X metals, those fish preferred Clark Fork River water with 0X metals 84% of the time (Table 10). When given the option of 4X water, rainbow trout significantly avoided it compared to the 1X water spending only 30% of their time in 4X as compared to 50% in the 1X.

Task 4: Acclimation to hard and soft tributary water -- Influence on avoidance/preference response. Measured metals concentration in the 1X test waters were near the nominal concentration for all metals, and the 0X was below or near detection limits (Table 11).

The water hardnesses used in this Task were Clark Fork River, 100; soft water tributary, 50; and hard water tributary, 200 mg/L (as $CaCO_3$). Rainbow trout did not show a preference or avoidance of Clark Fork River water (100 mg/L hardness) when

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acclimated to hardnesses of 50 and 200 mg/L in the absence of metals (Table 12). Rainbow trout acclimated to a soft water tributary spent 42% of their time in 0X Clark Fork River water; and when acclimated to a hard water tributary, spent 54% of their time in 0X Clark Fork River water. However, when metals were present (1X Clark Fork River water), fish significantly avoided them regardless of previous water hardness experience. When either soft or hard tributary water was used for acclimation and reference, rainbow trout would spent less than 4% of their time in 1X Clark Fork River water.

Interlaboratory validation. Using similar techniques and the same species, avoidance testing at the Columbia laboratory produced almost identical results for Task 1. Measurements for Cu and Zn were usually within 10% of the nominal concentration; whereas measured Cd concentrations were 30% above nominal concentrations, and measured Pb concentrations were 30% lower than nominal concentrations (Table 13). The 0.1X and 0.5X test waters contained Cd and Pb concentrations that were at or below the detection limits.

Brown trout in the Columbia study avoided Clark Fork River water when metals concentrations were 0.5X or greater (Table 14), identical results to the Jackson study. In both the Jackson and Columbia studies the threshold for brown trout avoidance was between 0.1X and 0.5X test concentrations. For the Columbia brown trout, total sec in test water was 50% for 0X test water, dropped to 33% for 0.1X, and dropped to less than 15% for 0.5X,

1X, 2X, and 4X test waters. The greatest avoidance was observed at 4X, 1X, and 2X when brown trout spent 10, 11, and 12%, respectively, of their time in the test water. But, a decrease in avoidance was observed with the higher concentration 10X test water (21% time in test water).

Testing with rainbow trout at Columbia was performed over a limited range of test waters (Table 15), but the results were similar to the findings at Jackson. Rainbow at Columbia avoided both the 0.5X and 1X test waters by spending 11 and 5.9% respectively, of their time in the test waters as compared to 46% for the 0X. Rainbow trout tested at Jackson spent 1.9 and 2.1% of their time in the 0.5X and 1X test waters.

DISCUSSION

A range of metals mixtures and pH values characteristic of Clark Fork River conditions resulted in avoidance by brown trout and rainbow trout (Figure 6 and 7). In the absence of reduced pH, threshold concentrations for inducing the avoidance reaction lies between 0.1 and 0.5 times the representative metals concentrations for brown trout and is below 0.1 for rainbow trout. Reduction in pH of the Clark Fork River has been observed after storm events (Glenn Phillips, unpublished records, Montana Fish, Wildlife, and Parks). This increase in acidity, even in the absence of metals, will result in avoidance by both brown trout and rainbow trout. The threshold pH for inducing the avoidance reaction is between pH 8.0 and 7.0 for both species. In contrast, Gunn and Noakes (1986) found that brook trout do not react to changes in pH until the water becomes quite acidic (pH 5.0). Our studies indicate the presence of increased acidity together with metals would not increase the avoidance response over that expected from metals alone.

When tested as a single metal, the literature on copper and zinc indicates an avoidance threshold which was similar to our findings for the combination of metals. The copper avoidance threshold for rainbow trout ranged from 0.1 to 7.3 ug/L (Folmar 1976, Giattina et al. 1982), and this range would include the 1.2 ug/L Cu concentration calculated in our 0.1X test water. Sprague (1964) found that young atlantic salmon could detect 2.3 ug/L copper. Rainbow trout avoided 5.6 ug/L Zn (Sprague 1968), but

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atlantic salmon had a Zn avoidance threshold of 53 ug/L (Sprague 1964). Our measured Zn in the 0.1X test water was 14 ug/L.

Sprague (1964) performed avoidance testing on a mixture of Cu and Zn and found the combination of the two metals reduced the avoidance threshold below that for each metal tested individually. In combination, 0.4 ug/L Cu and 6.1 ug/L Zn produced an avoidance reaction in atlantic salmon. This is 5 to 8 times lower than the concentration required to initiate avoidance from the individual thresholds of 2.3 ug/L Cu and 53 ug/L Zn.

We recently evaluated the avoidance response of cutthroat trout to Cd, Cu, Pb, and Zn which we tested individually and in a mixture (Dan Woodward, unpublished data, U.S. Fish and Wildlife Service). We used criteria concentrations for 50 mg/L hardness and the same procedures as this experiment. Cutthroat trout avoided Cu (6.5 ug/L) and Zn (55 ug/L) over 90% of the time when tested individually, but there was no avoidance of Cd (0.66 ug/L) and Pb (1.3 ug/L) when tested individually. When a 1X mixture was tested (all four metals, each at its criteria concentration), avoidance was greater than 90%. Therefore, avoidance of the mixture could be attributed to Cu and Zn, and the response to Cu and Zn in the mixture was not greater than that expected by either Cu or Zn acting individually.

Avoidance behavior to Cd and Pb has not been studied as extensively as Cu and Zn in salmonids. McNicol and Scherer (1991) reported that lake whitefish avoided Cd at a threshold of

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0.2 ug/L in a counter-current avoidance chamber. However, the avoidance response was only significant at less than 1 ug/L or greater than 8 ug/L. Giattina and Garton (1983) reported they have unpublished data defining an avoidance threshold of 26 ug/L for Pb and rainbow trout.

The avoidance response of rainbow trout was more sensitive than brown trout and may explain the absence of rainbow trout from the upper Clark Fork River. Giattina and Garton (1983) reported the difference in avoidance response between species can be explained by inherent activity level. In closely monitored studies on feeding behavior, we measured slower response time and less activity in brown trout when compared to rainbow trout (unpublished report on toxicity of metals in food-chain, D. Woodward, Jackson Protocol P92-40050-10-02). Therefore, the avoidance of metals by rainbow trout may be increased over that of brown trout by the greater activity level of rainbow trout.

In the highest metals concentration (10X), brown trout had a reduced avoidance response when compared to the 1X and 2X concentration. At high Cu concentrations perceptive acuity may be impaired due to injury of sensory tissue (Gardner and LaRoche 1973, Hara et al. 1976). If brown trout were slow to initially avoid the 10X concentrations in our experiments, injury to olfactory function may have affected their ability to make a future avoidance response. The presence of brown trout in the upper Clark Fork River may be explained through injury to the olfactory organ during short-term high metals concentrations,

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which would desensitize their sensory perception and interfere with their ability to detect and avoid lower metals concentrations.

Even after long-term acclimation to ambient 1X-metals Clark Fork River water, rainbow trout preferred Clark Fork River 0X water. Furthermore, rainbow trout acclimated to simulated soft or hard water tributaries did not show a preference or avoidance of medium hard Clark Fork River water when metals were absent. However, the presence of 1X metals in the Clark Fork River water resulted in a strong avoidance response. The consequence of these findings to rainbow trout populations in the Clark Fork River are two fold: 1) rainbow trout do not acclimate to the ambient metals concentrations in the Clark Fork River, and will always prefer low metals tributaries. And 2) recruitment of rainbow trout from low metal tributaries will be limited because of metals in the Clark Fork River.

Similarly, acclimation of rainbow trout to 13 ug/L Zn did not influence the 5.6 ug/L avoidance threshold (Sprague 1968). Rainbow trout pre-exposed to Cu identified and avoided a copper concentration of 6.4 ppb as compared to 7.3 ppb for unacclimated fish (Giattina et al. 1982). These two values were not significantly different, indicating a prior exposure to low concentrations of copper did not influence the avoidance threshold.

The surface water criteria for Cd, Cu, Pb, and Zn are based on hardness, frequency, and duration (USEPA 1987). Acute

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criteria are defined as one-hour average concentration which are not to be exceeded "more than once every 3 years" and the chronic criteria are defined as 4-d average concentration which are not to be exceeded "more than once ever 3 years". Acute and chronic equations were developed for each metal using hardness as a variable. Using Cu and 100 mg/L hardness as an example, the acute criteria is 18 ug/L and the chronic criteria is 12 ug/L. An increase in hardness would increase the criteria and a decrease in hardness would decrease the criteria.

In our experiment, the 1X concentration contained Cd, Cu, and Pb at the criteria concentration and Zn at 45% of the criteria concentration. Brown trout and rainbow trout spent only 13% and 2%, respectively, of their time in water containing the 1X; and 20% and 2% of their time in water containing the 0.5X concentration. Therefore, when the criteria concentration for these metals is exceeded, we can expect fish to avoid conditions in the Clark Fork River.

Frequency of exceedence of aquatic life criteria in the Clark Fork River have been recently summarized (See Surface Water Chapter). Since 1980 all 347 water samples taken from Silver Bow Creek exceeded both the acute and chronic Cu criteria, and all but one of 343 samples exceeded the acute and chronic Zn criteria. Exceedences of Cd and Pb criteria were less frequent in Silver Bow Creek. During the same time period, criteria exceedences in the Clark Fork River from Warm Springs to Milltown reservoir occurred in all reaches for all metals, although Cu

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criteria exceedences were more frequent and more severe. Exceedences of the chronic Cu criteria ranged from 19% of the samples in the Milltown to Rock Creek reach to 52% of the samples in the headwaters reach above Little Blackfoot Creek.

Avoidance experiments and metals monitoring studies suggest brown trout and rainbow trout would be repelled from the Clark Fork River and Silver Bow Creek above Milltown reservoir. Studies comparing trout numbers and biomasses in the upper Clark Fork River and Silver Bow Creek with reference sites are supportive of our findings (see Report of Assessment). There was a complete absence of trout from Silver Bow Creek. Reference sites contained an average of 36-fold more juvenile trout and 11-times more adult trout than did Clark Fork River sites. Reference sites were selected to be comparable with Clark Fork River sites on the basis of geology, land type, valley bottom type, and land and water uses. Adjustments were also made for habitat and flow differences. The only remaining variable between paired Clark Fork River sites and reference sites was metals contamination. Therefore, we believe the reduced standing crop of trout in the Clark Fork River and Silver Bow Creek is due in part to avoidance of metals contamination of surface water.

CONCLUSIONS

* A range of metals mixtures and pH values characteristic of
  Clark Fork River conditions resulted in avoidance by brown
  trout and rainbow trout.

* Threshold concentrations for inducing the avoidance reaction
  lies between 0.1 and 0.5 times the representative metals
  concentrations for brown trout, or > 0.11 < 0.55 ug/L for
  Cd; > 1.2 < 6 ug/L for Cu; > 0.32 < 1.6 ug/L for Pb; and > 5
  < 25 ug/L for Zn.

* Threshold concentrations for inducing the avoidance reaction
  is below 0.1 times the representative metals concentrations
  for rainbow trout, or < 0.11 ug/L for Cd; < 1.2 ug/L for Cu;
  < 0.32 ug/L for Pb; and < 5 ug/L for Zn.

* The threshold pH for inducing the avoidance reaction is
  between 7.0 and 8.0 for both brown trout and rainbow trout.

* Rainbow trout do not acclimate to the 1X representative metals
  concentrations in simulated Clark Fork River water, and will
  prefer low metals (0X) waters.

* Rainbow trout acclimated to low metals in either soft or hard
  water representative of some tributaries, will avoid
  simulated Clark Fork River water with 1X metals.

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Table 1. Mean (standard deviation in parenthesis) for measured concentrations of metals in filtered test water sampled at six different times during Task 1 at NFCRC - Jackson.

Species, test water	Zn	Pb	Cd	Cu
Brown trout				
0X	9.7 (4.5)	<1.7	<0.4	<1.2
0.1X	14 (2.3)	<1.7	<0.4	1.9 (1.2)
0.5X	32 (4.5)	<1.7	0.6 (0.1)	6.5 (0.3)
1X	51 (2.5)	4.4 (0.4)	1.3 (0.5)	11 (0.6)
2X	105 (7.2)	9.0 (0.8)	2.1 (0.1)	22 (0.8)
4X	213 (32)	17 (0.7)	4.2 (0.1)	50 (1.9)
10X	470 (33)	37 (2.9)	11 (0.4)	115 (4.0)
Rainbow trout				
0X	8.1 (3.5)	<1.7	<0.4	<1.2
0.1X	14 (5.1)	<1.7	<0.4	1.6 (0.3)
0.5X[a]	25 (1.2)	1.8 (1.0)	0.6 (0.0)	7.4 (0.5)
1X	54 (4.3)	3.1 (0.6)	1.0 (0.1)	11 (1.0)
2X	107 (4.3)	8.9 (1.1)	2.2 (0.1)	23 (1.0)
4X	195 (10)	17 (1.0)	4.3 (0.2)	47 (2.6)
10X	464 (12)	38 (1.4)	11 (0.7)	118 (2.4)

[a] n = 5

Table 2. Avoidance response of brown trout to different metals concentrations in test waters simulating the Clark Fork River, Task 1. Reference water was 0X, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Letter ([a]) indicates significantly different from 0X metals concentration ($P \leq 0.05$).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
0 X	601 (132)	50 (11)	61 (2.6)	11 (2.2)
0.1 X	545 (95)	45 (8.0)	58 (8.1)	10 (2.9)
0.5 X	245 (34)[a]	20 (2.8)	48 (6.4)	5.7 (1.9)[a]
1 X	161 (33)[a]	13 (2.8)	46 (9.6)	3.7 (0.4)[a]
2 X	96 (45)[a]	8.0 (3.7)	31 (6.8)[a]	3.0 (0.5)[a]
4 X	209 (87)[a]	17 (7.3)	40 (6.8)[a]	4.3 (1.3)[a]
10 X	333 (182)[a]	28 (15)	54 (26)	5.7 (1.9)[a]

Table 3. Avoidance response of rainbow trout to different metals concentrations in test waters simulating Clark Fork River water, Task 1. Reference water was 0X, and test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Letter (a) indicates significantly different from 0X avoidance response ($P \leq 0.05$).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
0X	625 (117)	52 (9.8)	67 (23)	12 (6.7)
0.1X	91 (42)a	7.6 (3.5)	38 (12)a	2.3 (0.77)a
0.5X	23 (6.6)a	1.9 (0.55)	19 (6.2)a	1.2 (0.25)a
1X	25 (11)a	2.1 (0.90)	19 (5.9)a	1.2 (0.30)a
2X	20 (7.9)a	1.6 (0.66)	18 (5.8)a	1.1 (0.27)a
4X	27 (11)a	2.2 (0.95)	20 (7.3)a	1.3 (0.37)a
10X	14 (8.4)a	1.1 (0.70)	13 (4.1)a	0.93 (0.23)a

Table 4. Mean (standard deviation in parenthesis) for measured concentrations of metals in filtered reference and test water sampled at six different times during Task 2B at NFCRC - Jackson.

Species, reference or test water		Zn	Pb	Cd	Cu
Brown trout					
8.0	0X[a]	<4.9	<1.7	<0.4	<1.2
5.0	1X	59 (5.5)	3.6 (0.8)	1.1 (0.0)	14 (1.1)
6.0	1X	54 (17.3)	2.9 (1.3)	0.9 (0.4)	11 (4.3)
7.0	1X	66 (16.3)	3.2 (0.7)	1.1 (0.2)	12 (2.0)
8.0	1X	56 (4.7)	3.3 (0.8)	1.1 (0.1)	13 (1.9)
Rainbow trout					
8.0	0X[a]	9.6 (5.2)	<1.7	<0.4	1.6 (1.1)
5.0	1X	57 (5.5)	3.2 (0.4)	1.0 (0.1)	14 (0.8)
6.0	1X	55 (7.9)	3.0 (0.6)	1.0 (0.0)	13 (1.0)
7.0	1X[b]	53 (4.0)	3.1 (0.7)	1.0 (0.0)	11 (0.1)
8.0	1X[b]	52 (6.1)	3.0 (0.8)	1.0 (0.0)	12 (1.5)

[a] n = 4

[b] n = 5

Table 5. Avoidance response of brown trout to test water having four different pH's and simulating the Clark Fork River, Task 2A. Reference water was pH of 8.0, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Values with common letters are not significantly different ($P \leq 0.05$, Tukey studentized range test).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
8.0	582 (96)[a]	48 (8.0)	57 (20)[a]	14 (7.1) [a]
7.0	202 (32) [b]	17 (2.6)	43 (3.6)[ab]	4.6 (0.9) [b]
6.0	81 (11) [c]	6.8 (0.9)	29 (2.5) [b]	2.8 (0.4) [c]
5.0	84 (14) [c]	7.0 (1.2)	29 (5.0) [b]	3.0 (0.5) [c]

Table 6. Avoidance response of brown trout to test water having four different pH's in combination with 1X metals concentrations in water simulating the Clark Fork River, Task 2B. Reference water was pH of 8.0, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Values with common letters are not significantly different (P≤0.05, Tukey studentized range test).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
8.0 1X	157 (82)	13 (6.8)	45 (12) [a]	3.5 (1.1)
7.0 1X	72 (23)	6.0 (1.9)	31 (5.6) [b]	2.4 (0.4)
6.0 1X	113 (106)	9.4 (8.8)	21 (3.9) [c]	4.8 (4.8)
5.0 1X	63 (21)	5.3 (1.8)	25 (6.3) [bc]	2.4 (0.38)

Table 7. Avoidance response of rainbow trout to test water having four different pH's and simulating the Clark Fork River, Task 2A. Reference water was pH of 8.0, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Values with common letters are not significantly different (P≤0.05, Tukey studentized range test).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
8.0	691 (113)[a]	57 (9.4)	62 (8.4)[a]	11 (2.5)[a]
7.0	137 (40)[b]	11 (3.3)	36 (4.8)[b]	3.8 (1.2)[b]
6.0	45 (5.1)[c]	3.8 (0.42)	29 (3.3)[bc]	1.5 (0.15)[c]
5.0	28 (5.5)[d]	2.3 (0.46)	23 (4.4)[c]	1.2 (0.06)[c]

Table 8. Avoidance response of rainbow trout to test water having four different pH's in combination with 1X metals concentration in water simulating the Clark Fork River, Task 2B. Reference water was pH of 8.0, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Values with common letters are not significantly different ($P \leq 0.05$, Tukey studentized range test).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
8.0 1X	62 (11)[a]	5.2 (0.95)	38 (5.4)[a]	1.6 (0.11)
7.0 1X	52 (17)[a]	4.3 (1.4)	31 (8.3)[b]	1.6 (0.28)
6.0 1X	35 (12)[b]	2.9 (0.96)	28 (7.4)[b]	1.2 (0.12)
5.0 1X	36 (14)[b]	3.0 (1.2)	27 (5.3)[b]	1.3 (0.29)

Table 9. Mean (standard deviation in parenthesis) for measured concentrations of metals in filtered water sampled from the test water (n = 6), the reference water (n = 5), and the acclimation water (n = 18) of Task 3 at NFCRC - Jackson.

Test phase, test water	Zn	Pb	Cd	Cu
Reference water				
1X	50 (3.2)	4.0 (0.7)	1.0 (0.1)	11 (0.7)
Test water				
0X	<4.9	<1.7	<0.4	<1.2
1X	53 (4.2)	4.1 (0.4)	1.0 (0.1)	11 (0.6)
4X	187 (6.5)	17 (0.3)	4.4 (0.1)	47 (3.2)
Acclimation water				
1X	53 (12.4)	2.1 (1.3)	0.8 (0.1)	12 (0.5)

Table 10.   Avoidance response of rainbow trout to test waters of different metals concentrations after 30 d acclimation to simulated Clark Fork River water with 1X metals, Task 3.   Reference water was 1X, and test time was 1200 sec.   Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests.   Values with common letters are not significantly different (P≤0.05, Tukey studentized range test).

Test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
0X	1010 (84)a	84 (7.0)	45 (11)a	25 (3.3)a
1X	598 (72)b	50 (6.0)	85 (13)b	7.6 (1.6)b
4X	358 (96)c	30 (8.0)	73 (13)b	4.8 (1.4)c

Table 11. Mean (standard deviation in parenthesis) for measured concentrations of metals in filtered test water sampled at six different times during Task 4 at NFCRC - Jackson.

Water hardness, test water	Zn	Pb	Cd	Cu
Soft water reference				
0X	13 (10)	<1.7	<0.4	<1.2
1X	52 (5.2)	2.9 (0.5)	1.2 (0.1)	12 (0.9)
Hard water reference				
0X	6.4 (5.3)	<1.7	<0.4	<1.2
1X	57 (16)	2.7 (0.4)	1.1 (0.1)	11 (0.6)

Table 12. Avoidance response of rainbow trout to test water of different metals concentrations and simulating the Clark Fork River (CFR), Task 4. Acclimation and reference water was OX in either hard or soft water, and test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 6 with each n based on 4 avoidance tests. Values with common letters are not significantly different (P≤0.05, Tukey studentized range test).

Water hardness, test water	Total time in test water (sec)	Percent time in test water	Number of trips into test water	Trip time (sec)
Soft water reference				
OX CFR	498 (92)a	42 (7.7)	64 (5.4)a	8.6 (1.8)a
1X CFR	46 (14)b	3.8 (1.2)	·29 (6.3)b	1.5 (0.18)b
Hard water reference				
OX CFR	644 (108)a	54 (9.0)	51 (6.5)a	18 (10)c
1X CFR	43 (15)b	3.6 (1.3)	18 (5.3)c	2.7 (1.0)d

Table 13. Mean (standard deviations in parenthesis) for measured concentrations of metals in filtered test water sampled during the interlaboratory validation task, NFCRC - Columbia.

Species, test water		N	Zn	Pb	Cd	Cu
Brown trout	N					
0	X	4	< 4.9	<1.7	<0.4	1.8 (1.2)
0.1	X	4	8.2 (2.5)	<1.7	<0.4	2.6 (0.4)
0.5	X	4	30 (9.5)	<1.7	0.7 (0.05)	6.4 (0.5)
1	X	4	47 (2.8)	1.8 (0.2)	1.4 (0)	13 (2.9)
2	X	4	87 (5.5)	5.9 (1.4)	3.0 (0.1)	20 (0.6)
4	X	4	179 (2.2)	8.0 (0.4)	6.0 (0.5)	49 (2.7)
10	X	3	424 (26)	21 (1.6)	19 (0.6)	129 (8.4)
Rainbow trout	N					
0	X	3	6.9 (3.0)	<1.7	<0.4	2.6 (0.4)
0.5	X	3	29 (3.4)	<1.7	0.6 (0.1)	6.8 (0.9)
1	X	3	51 (1.9)	2.2 (1.0)	1.2 (0.1)	11 (0.5)

Table 14. Avoidance response of brown trout to different metals concentrations in simulated Clark Fork River water as identified in the interlaboratory validation task, NFCRC - Columbia; X (in $\mu g/L$) = 1.1 Cd, 12 Cu, 3.2 Pb, and 50 Zn. Alternative choice test water was 0X, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 12 with each n based on a single avoidance test with two fish per chamber. Letter ([a]) indicates significantly different from 0X metals concentration (P≤0.05, Tukey's).

Metals concentration	Percent time in treatment	Number of trips into treatment	Trip time (sec)
0 X	50 (14)	63 (15)	11 (5.1)
0.1 X	33 (20)	55 (15)	8.1 (6.6)
0.5 X	15 (7.9)[a]	48 (15)	5.8 (7.5)[a]
1 X	11 (8.9)[a]	34 (20)[a]	3.4 (0.9)[a]
2 X	12 (8.0)[a]	34 (9.8)[a]	3.9 (1.8)[a]
4 X	10 (7.5)[a]	30 (12)[a]	3.9 (2.2)[a]
10 X	21 (23)[a]	27 (13)[a]	7.7 (7.1)

Table 15. Avoidance response of rainbow trout to different metals concentrations in simulated Clark Fork River water as identified in the interlaboratory validation task, NFCRC – Columbia; X (in μg/L) = 1.1 Cd, 12 Cu, 3.2 Pb, and 50 Zn. Alternative choice test water was 0X, and total test time was 1200 sec. Values are means (standard deviation in parenthesis), n = 12 with each n based on a single avoidance test with one fish per chamber. Letter (^{a}) indicates significantly different from 0X metals concentration (P$\leq$0.05, Tukey's).

Metals concentration	Percent time in treatment	Number of trips into treatment	Trip time (sec)
0 X	46 (19)	69 (25)	9.4 (5.8)
0.5 X	11 (7.5)[a]	48 (27)	2.7 (1.5)[a]
1 X	5.9 (3.2)[a]	39 (17)[a]	1.8 (0.5)[a]

Water Supply Tanks

| 1B | 2B | 3B | 4B |

| 1C | | 3C |
| 2C | | 4C |

Avoidance Chamber

Mixing Box

Water Supply Tanks

| 3A | 1A |
| 4A | 2A |
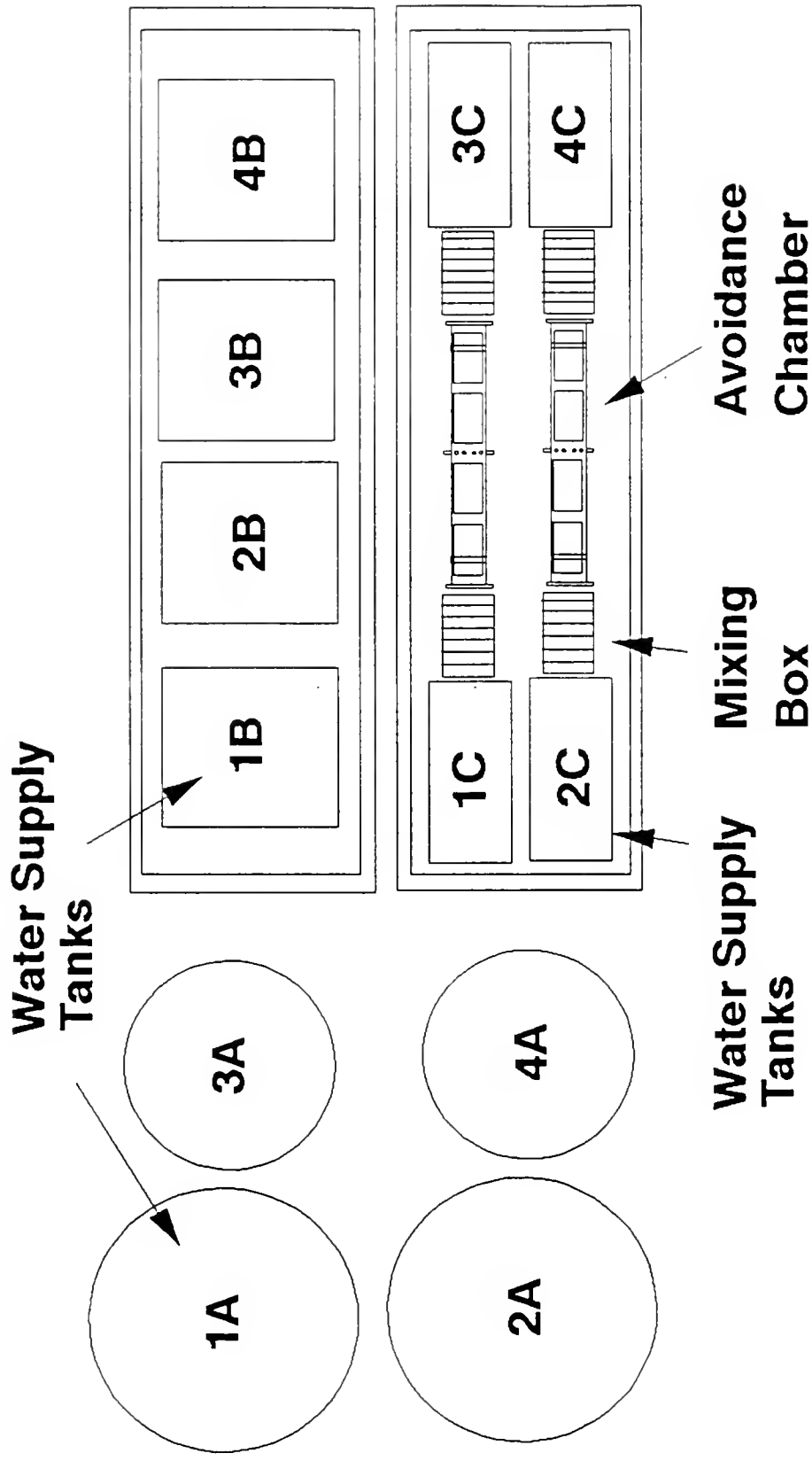
Figure 1. Arrangement of two avoidance chambers, mixing boxes, and water supply tanks (series A, B, and C) used in water control and avoidance testing.
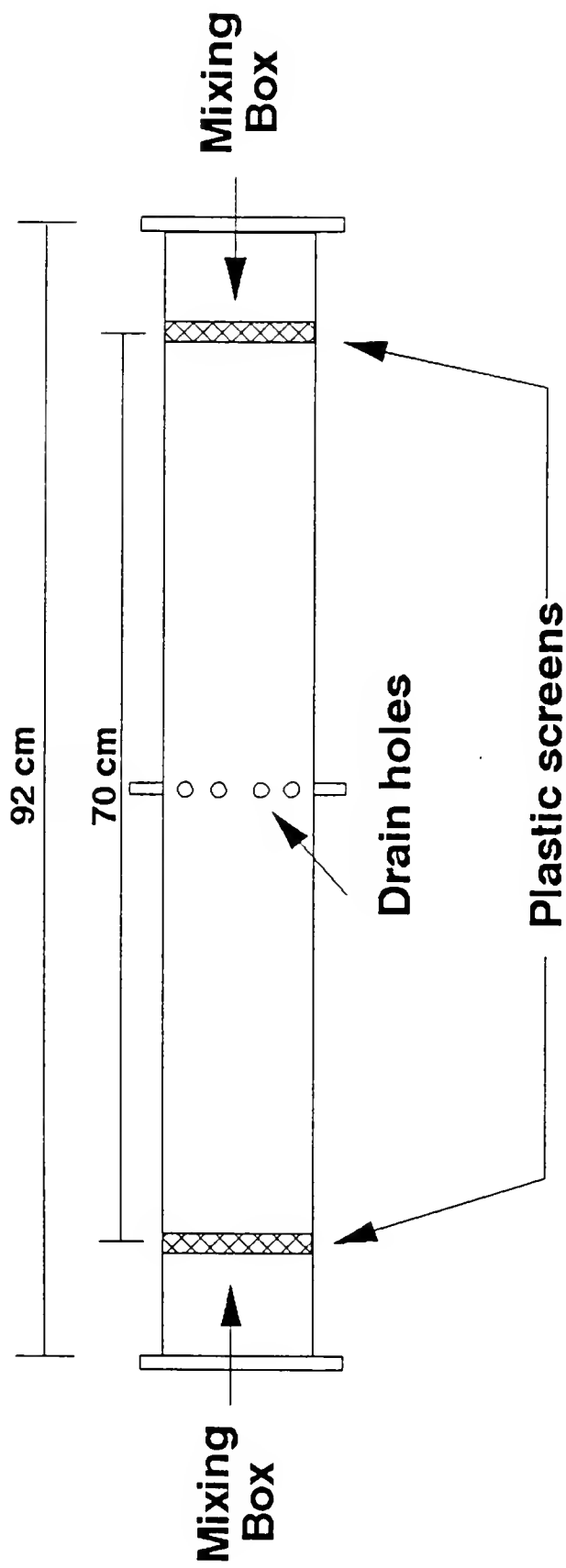
Figure 2. Avoidance chamber. Flow enters from each mixing box and exits at center drain holes Chamber diameter is 11 cm.

Figure 3. Schedule for testing three, four, and seven treatments when testing two treatments a day and six trials per treatment. Follows a balanced incomplete block design (Cochran and Cox 1957).
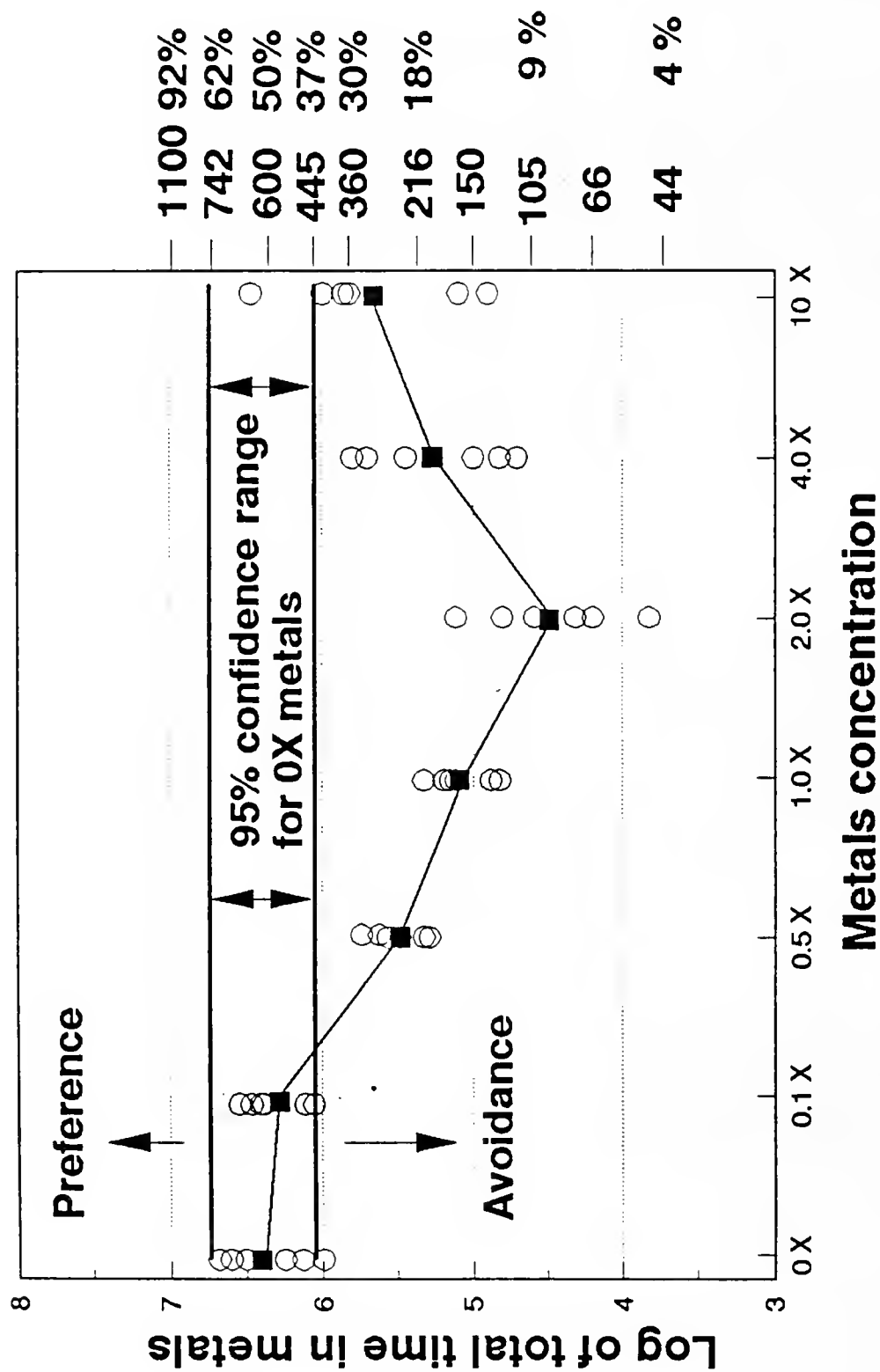
Figure 4. Avoidance/preference response of brown trout to metals in Task 1 using log of total time in test water end of avoidance chamber versus metals concentration. Actual time (sec) in test water end and percent time in test water are also shown. ■ = mean ○ = each trial
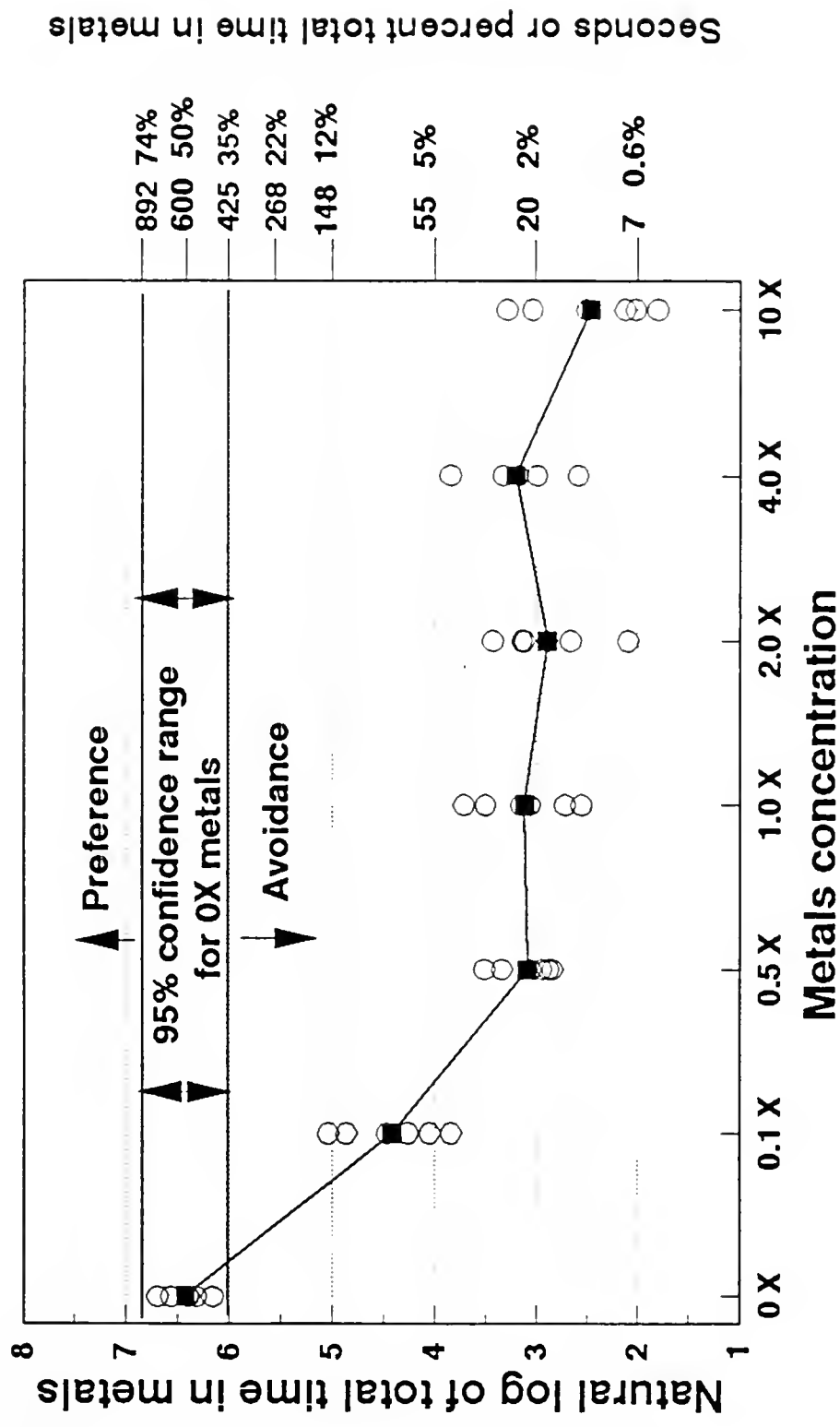
Figure 5. Avoidance/preference response of rainbow trout to metals in Task 1 using log of total time in test water end of avoidance chamber versus metals concentration. Actual time (sec) in test water end and percent time in test water end are also shown. ■ = mean ◯ = each trial
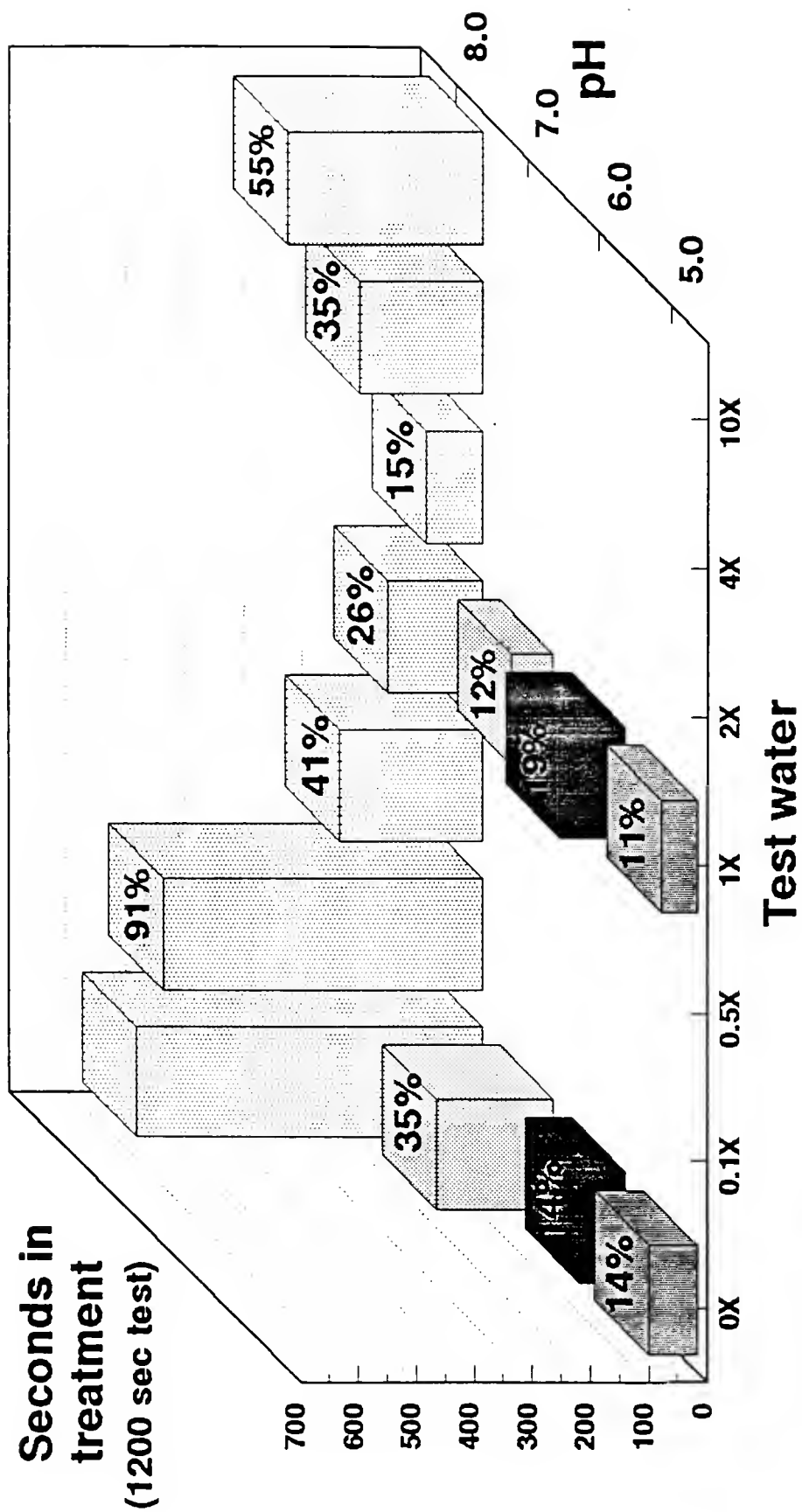
Figure 6. Response of brown trout to metals and acidity presented separately and together In Task 1 and 2. Values and column heights are expressed as percentage of control response.
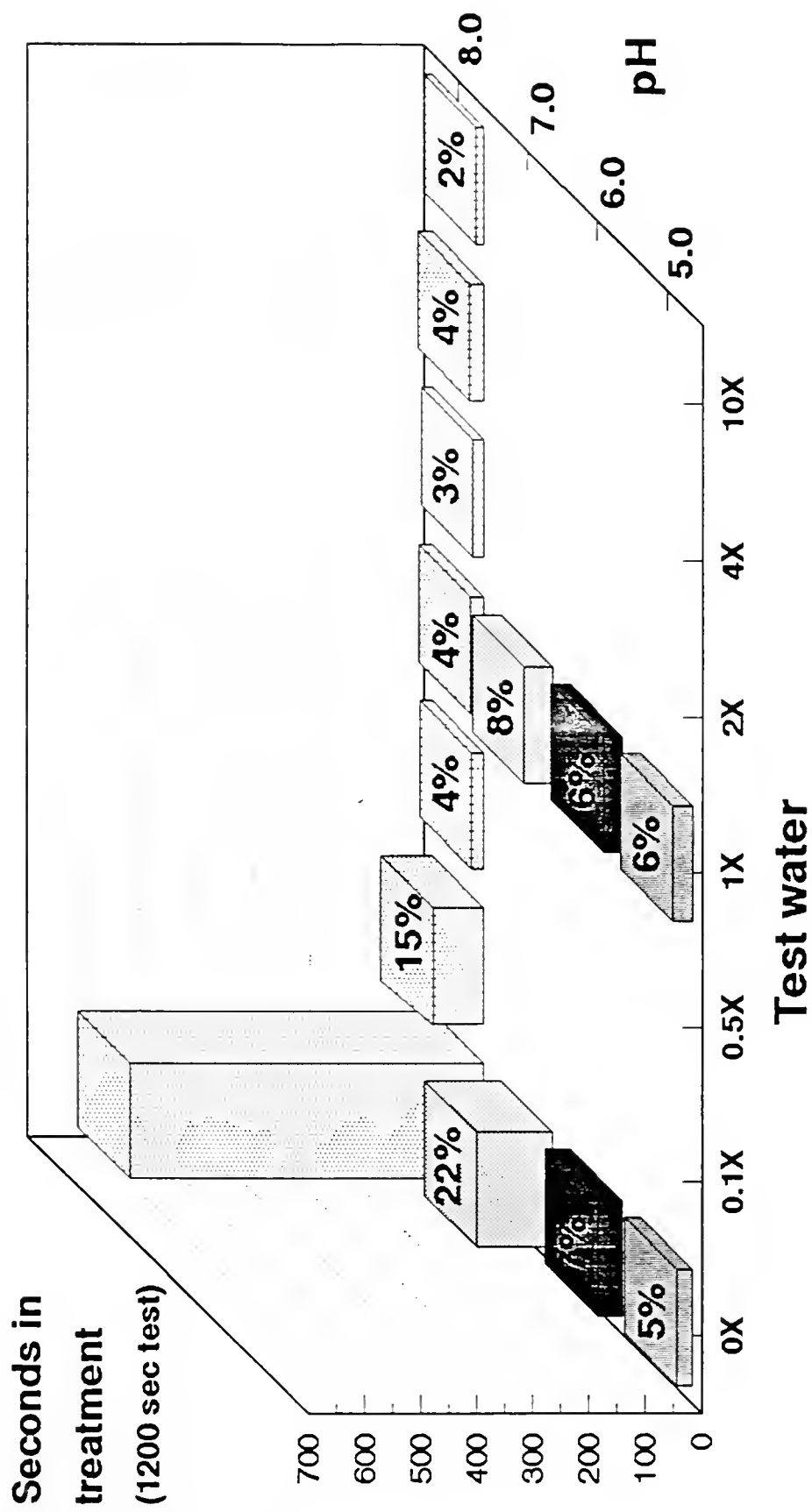
Figure 7. Response of rainbow trout to metals and acidity presented separately and together in Task 1 and 2. Values and column heights are expressed as percentage of control response.

AQUATIC RESOURCES INJURY REPORT

APPENDIX E

Chronic Toxicity of Cadmium, Copper, Lead, and Zinc to Rainbow Trout and Brown Trout at Concentrations and Forms Present in Water and Aquatic Invertebrate Food Chains in the Upper Clark Fork River

Prepared by:

Dan Woodward, U.S. Fish and Wildlife Service
Harold Bergman, University of Wyoming
Charles Smith, U.S. Fish and Wildlife Service

Chronic Toxicity of Cadmium, Copper, Lead, and Zinc to Rainbow Trout and Brown Trout at Concentrations and Forms Present in Water and Aquatic Invertebrate Food Chains in the Upper Clark Fork River.

D.F. Woodward, H.L. Bergman, and C.E. Smith

INTRODUCTION

In the Clark Fork River metals are available to benthic organisms and fish through uptake of dissolved forms across the gill and by assimilation through the food chain (Lee et al. 1984; Swartz et al. 1985). Concentrations of Cd, Cu, and Pb in aquatic insects in the Clark Fork are 2-14 times greater than concentrations in the same taxa from less-contaminated tributaries (Luoma et al. 1989). Benthic invertebrates are important as food sources for fish and waterfowl and occupy an essential niche in trophic energy transfer and nutrient cycling. Thus, fish and other vertebrates feeding at higher trophic levels may be chronically exposed to metals through both food chain and water.

Food-chain uptake and aqueous exposure should both be considered when evaluating the risk of metals contamination to organisms in the Clark Fork River. Detrital- and filter-feeding invertebrates may accumulate more metals by ingestion than from water (Luoma 1989). Metal assimilation from ingested particles may be less efficient than that from water, but it can be significant if the food source has high concentrations of metals. Studies have indicated that dietary uptake of Cd, Cu, Co, Pb, and

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Zn is a predominant pathway of metal accumulation (Crespo et al.
1986; Wekell et al. 1986; Dallinger et al. 1987; Pratap et al.
1989). At sublethal dietary levels, Cd interfered with calcium
(Ca) and magnesium (Mg) metabolism in tilapia _Oreochromis_
mossambicus (Pratap et al. 1989), and Cu reduced growth in
rainbow trout _Oncorhychus_ _mykiss_ (Lanno et al. 1985; Julshamn et
al. 1988). Morphological and functional alteration were induced
in rainbow trout intestine by dietary Cd and Pb (Crespo et al.
1986).

The objective of this study was to determine effects on
rainbow trout and brown trout _Salmo_ _trutta_ for a combination of
trace metals interacting together through both aqueous and
dietary exposure.

METHODS

Experimental fish -- Eyed embryos of rainbow trout and brown trout were obtained in the fall of 1991 from the Ennis National Fish Hatchery, Montana; and the Saratoga National Fish Hatchery, Wyoming. Eggs were held in Heath[R] incubators until hatching. Temperature was maintained at 10°C during holding and testing. Eyed embryos, larvae, and juveniles were handled so as to minimize stress in accordance with the NFCRC-Columbia Animal Welfare Plan, and the Region 6 Fish Health Policy.

Exposure water -- Test and control water were formulated at the Jackson Field Station to simulate conditions in the Clark Fork River during spring conditions (hardness, 100 mg/L; alkalinity, 100 mg/L; pH, 7.2 - 7.8). Water was analyzed daily to insure that hardness, alkalinity, conductivity, and pH were within 5% of the experimental design. Test water contained a 1X concentration of metals; where 1X = 1.1 ug/L cadmium, 12 ug/L copper, 3.2 ug/L lead, and 50 ug/L zinc. The 1X metals concentration corresponds to concentrations measured in the Clark Fork River (Lambing 1991); and Cd, Cu, and Pb are also at ambient water criteria (USEPA 1987). No metals were added to the control water, referred to as 0X.

Exposure diets -- Benthic macroinvertebrates were collected from the upper Clark Fork River (Montana) in the spring of 1991 by personnel from the U.S. Fish and Wildlife Service and the Montana Department of Fish, Wildlife, and Parks. The collection sites (Figure 1) were 2 km below Warm Springs Creek (WS), 5 km

below Gold Creek (GC), and 2 km above Turah Bridge (TB). These sites were selected to represent a gradient in metals concentrations in benthic macroinvertebrates with downstream distance from the metals. Suspected contaminants associated with food chain organisms from these sites were As, Cd, Cu, Pb, and Zn. The TB site is approximately 200 km downstream from the source, and the invertebrates collected from this station were used as the reference diet.

Invertebrates from the three locations were frozen immediately after collection and transferred to the Fish Technology Center, Bozeman, Montana where they were prepared into dry diets. Procedures described by Woodward et al. (1993) were used to eliminate disease potential from the food organisms and to assure that the proper vitamins and minerals were present. Invertebrates collected from WS, GC, and TB were pasteurized and pelletized with a vitamin and mineral supplement added similar to procedures used in standard fish feed formulation (Piper et al. 1982). Food sources were pasteurized at 75° C for 15 min, and a 3% vitamin and mineral premix was added along with 2% binder. The diets were taken through an extrusion process to produce a #1 starter pellet. After processing, the diets were dried with an ambient 21° C forced-air drier.

Experimental procedure -- For both species, 75 newly hatched alevins were exposed until 88 days after hatching to 1X and 0X water exposure. The 0X and 1X water treatments were assigned in an alternating arrangement to 12 tanks. There were four

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identical cells within each tank which were assigned two each to
rainbow trout and brown trout by using a random numbers table.
Within a water treatment and species, the three diet treatments
(WS, GC, TB) were assigned to the cells at random four separate
times to achieve four replicates of each experimental variable
(two water exposures, two species, and three diets) for a total
of 48 experimental cells. Dietary exposures started at exogenous
feeding (26 d, brown trout; 18 d, rainbow trout) and continued to
88 days post hatch.

Exposures were conducted in a flow-through proportional
diluter system designed to deliver 1L of the appropriate exposure
water to each of the 12 water treatments. Each experimental cell
contained 4.2 L of exposure water and received 10 volume
additions per day. The appropriate concentration of each
exposure was maintained by an automated pipetting system
(Micromedic^R).

Biological measurements. - For each experimental cell, brown
trout were thinned to 40 fish at 26 d and 25 fish at 52 d;
rainbow trout were thinned to 40 fish at 18 d and 25 fish at 53
d. At each thinning and at 88 d, measurements of length (mm) and
weight (mg) were made on the individual fish removed.
Experimental cells were checked daily for mortality and
observations on behavior.

Measurement of feeding behavior followed procedures used in
previous studies (Woodward et al. 1989, 1993). A 5-min video
recording of each experimental unit was used to analyze fish

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behavior and included a 2-min non-feeding period, followed by a 3-min feeding period. At selected intervals during the study, overhead video recordings were made of a group of 5-7 fish from each experimental unit. At this time the fish were temporarily isolated within a 13- x 13- x 15-cm-area of the test chamber with a glass partition. During analysis of the recordings, the chamber was divided into 16 equal cells using a transparent grid (4 X 4) overlay placed over the video image. Feeding behavior was evaluated by counting the number of strikes directed toward the experimental diet during a 2-min segment of the recording.

At the termination of the test, eight fish of each species were selected for histopathological examination from each of the 6 combinations of water and diet exposures. Whole fish were placed in Bouin's fixative for 24 h, then transferred to 70% ethanol. After dehydration and clearing, whole fish were embedded in paraffin and sectioned at 4 um. Sections were mounted on slides stained with hematoxylin and eosin or rhodamine. Gill, liver, kidney, gastrointestinal tract, and pancreas were qualitatively analyzed for general pathology. At least five slides of each fish (four sections per slide) were analyzed.

Lipid Peroxidation. - At the termination of the test, eight fish were sampled from each of the 48 experimental cells for measurements of lipid peroxidation and tissue metal concentrations. These samples were immediately frozen in liquid nitrogen and stored at -70° C. At a later date, each frozen

sample was ground by mortar and pestle cooled with liquid nitrogen. About 200 mg of ground tissue was processed for measurement of lipid peroxidation.

The fluorometric assay described by Dillard and Tappel (1984) and Fletcher et. al (1973) was used to measure products of lipid peroxidation. A chloroform-methanol extraction of tissue was followed by the fluorometric measurement of products from lipid peroxidation. The 200 mg of ground sample was put in a glass homogenizer and a 2:1 mixture of HPLC grade chloroform:methanol was added at 35 times the weight of the sample (i.e. 7.00 mls for a 200 mg sample). The tissue was put through four passes in the homogenizer, an equal volume of water was then added followed by four additional passes in the homogenizer. The mixture was then vortexed for 2 min and poured into a corex tube. The mixture was spun at 3,000 rpm for 1 min and the chloroform layer was removed and measured at a wavelength of 340 excitation with a 425 emission wave length. Previous measurements demonstrated that these wave lengths were optimal.

Autopsy assessment. - Each fish was evaluated for unusual external characteristics when length and weight measurements were taken at 88 d post hatch. These characteristics included changes

in shape, color, and texture of the eyes, gills, head, fins, and body. At lease two fish from each experimental unit were given a complete autopsy inspection (Goede 1989). These fish were dissected and the gill, spleen, kidney, liver, and bile were

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evaluated along with fat content associated with each organ. Livers for an additional 5-10 fish were evaluated for metals residue and abnormalities.

Chemical analysis, water. - Filtered water samples were taken weekly from the 12 water treatments for metal determinations. Water was filtered using a Nalgene® 300 filter holder with a polycarbonate, 0.4 μM-pore-membrane. Filtered samples (100 mL each) were transferred to precleaned, 125-mL I-Chem® polyethylene bottles and preserved by addition of 1 mL Ultrex-II® nitric acid. Cadmium, Cu, Pb, and Zn were measured using a Perken-Elmer graphite furnace atomic absorption spectrophotometry (AAS), except Zn at the 1X treatment which was determined by flame AAS.

At two different times during the experiment, filtered water samples were collected from one of the 0X/WS experimental units at 15, 30, 45, 60, and 120 min after feeding to determine if metals in the diet had an effect on measured concentration of metals in the water. Measurements for Cd, Cu, Pb, and Zn were not elevated above background. Therefore, when feeding the WS diet, high concentrations of metals in food did not increase dissolved metal concentration in the water column.

Chemical analysis, fish and diet. - Fish and diet samples were collected during the study for metal analysis. Fish were collected at thinning and 88 d. Each diet source was sampled four times during the study for metals analysis. Three additional diet samples were collected before the start of the

study to determine percent moisture, protein, fat, and ash.

Fish were not fed 24 h before sampling. Whole body and liver samples from the autopsy assessment were stored in plastic bags at -25°C until analysis. Diets were "scanned" for aluminum (Al), arsenic As, barium (Ba), boron (B), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), silver (Ag), thallium (Ti), titanium (Ti), vanadium (V), and zinc (Zn). Based on the results of the scan and past experience (Woodward et al. 1993), five elements were selected for subsequent analysis of the diets: As, Cd, Cu, Pb, and Zn; and four elements were analyzed in fish tissue: As, Cd, Cu, and Pb. The method detection limits for these metals in ug/g were as follows: As, 0.74; Cd, 0.27; Cu, 0.31; and Pb, 0.88.

Fish and diet were analyzed with a inductively coupled plasma - mass spectroscopy (ICP-MS) on a Perkin Elmer Elan 5000 using USEPA method 200.8. Whole body and liver tissues were lyophilized, digested in a microwave and taken up to a 50 ml final volume in a 5% HNO_3 matrix. The samples were diluted at the instrument and an internal standard solution containing yttrium (Y), indium (In), and bismuth (Bi) were added to each sample at time of dilution to give an equivalent concentration of 50 ug/L of each internal standard element. Although this concentration is smaller than recommended in the method, the procedure was adequate for monitoring interferences and minimized the effect of impurities in internal standard element reagents.

Initial calibration verification, continuing calibration

verification, low level (CDRL) standard verification, and spike and duplicate measurements were used to verify the analytical method and followed procedures outlined by the manufacturer. Analytical duplicates and spikes were run every ten samples and the spikes were prepared by adding 1 ml of spike concentrate to 9 ml of the diluted digestate to give a final spike addition of 100 ug/L. No appropriate Laboratory Control Sample existed so SRM tissues were submitted as regular samples as an additional form of verification.

Data analysis and statistics. - Percent survival, growth, physiology, histopathology, and behavioral data were statistically evaluated using analysis of variance (ANOVA). Percent data were arc sine and square-root transformed, and behavioral count data were square-root transformed before analysis. The experiment was treated as a completely randomized, two-factor, split-plot design with four replicates. The statistical model included aqueous metal concentration as the main-plot treatment effect and dietary metal concentration and the interaction of aqueous and dietary metals as subplot effects (Cochran and Cox 1957). Means were compared using the least squares means test. Statistical significance in all tests was assigned at the $P \leq 0.05$ level.

Chemical analyses of water.

Except for Zn, measurements of all metals in OX water were below the detection limits of the method (Table 1). Mean of the measured concentrations in the 1X water were within 20% of the nominal value for Cu, Pb, and Zn. Measurements for Cd were 70-85% of the nominal value.

Chemical analyses of diets.

Analysis of the three diets indicated they were similar in protein, fat, moisture, and ash (Table 2). Protein was between 40-50% and lipid was greater than 15%; both were at or above the recommended limits required for salmonid starter diets at our water temperature (Piper et al. 1982). Food energy available for maintenance and growth is usually expressed as kilogram calories (kcal). From a gram each of protein, fat, and carbohydrate, there is 3.9, 8.0, and 1.6 kcal of energy available (Piper et al. 1982). Using the values in Table 2 for protein and fat, we can calculate the available energy for each diet -- 324 kcal in the WS diet, 315 kcal in the TB diet, and 288 in the GC diet. These values represent the energy available for maintenance and growth, and the two test diets -- WS and GC -- are within 8.5% of the TB reference diet.

When compared to the TB diet, As was about 3 times higher in the GC and WS diets; and Cu and Pb were about 2 times higher in the GC and WS diets (Table 2). Cadmium was elevated in the WS diet, but not detected in the GC and TB diet.

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<u>Survival and growth</u>.

Survival of brown trout and rainbow trout was not significantly affected by any combination of water or dietary exposure (Table 3 and 4). Relative to the 0X/TB exposure at 88 d, weight of brown trout was reduced 25% when exposed to metals in water alone (1X/TB) and was reduced 40% when exposed to metals in diet alone (0X/WS and 0X/GC, Table 5). Exposure to both water and diet (1X/WS and 1X/GC) resulted in a 50% reduction in weight compared to 0X/TB. Decreased weight was significant at days 26, 52, and 88; while a decrease in length was observed at days 52 and 88.

Rainbow trout growth to 88 d was not affected by exposure to 1X water, but exposure to either GC or WS diet resulted in a 40-50% reduction in weight at 53 d and 88 d when compared to fish or the TB diet and the same water (Table 6, Figure 2). Length of rainbow trout was reduced about 20% when exposed to GC or WS diet, but was unaffected by exposure to 1X water.

<u>Behavior</u>.

Despite large reductions in growth, video observations of brown trout feeding did not reveal any noticeable alteration or impairment of feeding behavior. Strike frequencies of brown trout were uniformly low (compared to rainbow trout) in all treatments throughout the duration of the study (Figure 3A). Low strike frequencies among brown trout were a result of long periods of latency between the introduction of the experimental diet and the initiation of feeding activity. Brown trout were

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characteristically slower to begin feeding and the 2-min observation period was not sufficient to monitor the feeding response and detect changes in feeding behavior.

Rainbow trout, on the other hand, fed aggressively with little or no latency between the introduction of food and feeding. The 2-min observation period was adequate to document altered feeding behavior in rainbow trout. Feeding frequencies of all rainbow trout averaged near 20 strikes/2 min on day 28, with trout exposed to 1X aqueous metals having slightly higher strike frequencies than those exposed to uncontaminated water (Figure 3B). Average feeding activity of TB fed fish steadily increased over the duration of the experiment, from less than 20 strikes to 50 strikes/2 min, while feeding activity of fish fed the GC and WS diets remained nearly constant over the duration of the experiment. By day 70 and 84 of exposure feeding behavior appeared to be strongly affected by dietary metal exposure. Rainbow trout fed the GC and WS diets exhibited greater than 50% reductions in feeding activity when compared to fish fed the TB diet.

Since food consumption increases with fish size, differences in growth and fish size, and not altered feeding behavior, may account for the observed differences in feeding frequency among rainbow trout fed different experimental diets. However, fish fed the GC and WS diets not only had drastically reduced feeding activity when compared to TB-fed fish, but feeding activity among these fish did not increase from 28 d through 84 d even though

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weight of these fish increased by as much as 8 fold over the same period. Therefore reduced feeding activity in rainbow trout fed the GC and WS diets, may have been due to poor acceptance of those diets; or the health status of fish on the WS and GC diets may have reduced their demand for feeding.

Bioaccumulation of metals.

Copper: For both species, 1X treatment had no effect on Cu tissue concentrations at day 88 (0X/TB VS 1X/TB) (Table 7 and 8). Within the 0X and 1X water exposures, concentrations of Cu in tissues of brown trout after 88 d were significantly higher by 2-3 fold for the GC and WS dietary treatments when compared to fish fed the TB diet (0X/TB VS 0X/GC and 0X/WS; 1X/TB VS 1X/GC and 1X/WS). With increased time of exposure (26, 52, and 88 days), Cu concentrations in brown trout continued to increase for all treatments. However, most of the increase was between day 52 and 88.

There were significant increases in tissue Cu of rainbow trout at 53 d due to the GC and WS diets. However, at 88 d, there were no significant increases in tissue Cu in rainbow trout due to water or diet exposure. The lower concentration at 88 d can probably be explained by the reduction in feeding activity in the WS and GC treatments as compared to TB.

Arsenic: Arsenic was not present in the 1X water exposure, and as could be expected, did not accumulate in tissue of either species when exposure occurred to water alone (Table 9 and 10). However, brown trout (at day 52 and 88) and rainbow trout (at day

53 and 88) accumulated significant amounts of As when receiving the GC and WS diets (0X/TB VS 0X/GC and 0X/WS; 1X/TB VS 1X/GC and 1X/WS). The magnitude of increase was 3 to 4 fold in brown trout at 88 days and was greater in brown trout than in rainbow trout.

As with Cu, increased time of exposure resulted in increased tissue As concentrations for brown trout and rainbow trout exposed to the GC and WS diets. However, the rate of tissue As increase in rainbow trout was less between days 53 and 88 than between days 18 and 53, and can probably be accounted for by the reduced feeding activity after 53 d by rainbow trout on the GC and WS diets.

Lead: Increases in tissue Pb due to water exposure at 88 days were observed in brown trout (0X/TB VS 1X/TB; 0X/GC VS 1X/GC) (Table 11). Exposing brown trout for 88 days to GC and WS diets resulted in significant increases in tissue Pb within the 0X and 1X waters (0X/TB VS 0X/GC and 0X/WS; 1X/TB VS 1X/GC and 1X/WS). Rainbow trout exposed over the same time period exhibited no tissue Pb increases (Table 12).

Cadmium: Exposure of both brown trout and rainbow trout to 1X water resulted in significant increases in tissue Cd at day 88 (0X/TB VS 1X/TB) (Table 13 and 14). Exposure to the GC and WS diet resulted in a significant additional Cd tissue concentration over that of 1X water alone at 88 d for brown trout (1X/TB VS 1X/GC and 1X/WS). Rainbow trout did not accumulate Cd through the diet.

Liver: Accumulation of metals in liver was more variable

than whole body tissue. There were 2-4 fold increases in Cu, As, Cd, and Pb in brown trout livers when exposed to both water and diet (0X/TB VS 1X/GC and 1X/WS)(Table 7), but these differences were not significant. Rainbow trout feeding on the GC and WS diet had a significant 3-fold increase in liver As (0X/TB VS 0X/WS and 0X/GC; 1X/TB VS 1X/GC and 1X/WS) (Table 10).

Health Assessment.

Histology: The most significant histological finding for brown trout was noted in exocrine pancreatic tissue of fish fed the diet from WS and GC. When compared with fish fed the 0X/TB diet, zymogen granules, precursors of digestive enzymes and commonly found in pancreatic cells (Figure 4), were lacking in 7 of 8 fish fed the 0X/WS diet (Figure 5) and 3 of 8 fish on the 1X/WS diet. The remaining 5 fish from the 1X/WS diet had reduced amounts of zymogen in their pancreatic cells. Swelling and vacuolar degeneration of some pancreatic cells were also noted in fish fed the 0X/WS and 1X/WS diets.

While degenerative changes consisting of vacuolation and sloughing of intestinal mucosal epithelial cells were seen in brown trout from all treatments they were more severe in fish from the 0X/GC and 1X/GC treatments (Figure 6). Very few differences were noted in other tissues between treatment groups and copper storage was not apparent in any livers.

The effects observed on brown trout pancreas and gut epithelium were not observed for any treatments involving rainbow trout. The most apparent histological difference between

treatment groups of rainbow trout were in livers between the OX and 1X waters. Within a diet, livers from fish in the 1X waters exhibited some degeneration of individual hepatocytes and reduced glycogen vacuolation when compared with fish from OX water.

Lipid peroxidation: An increased relative intensity measurement correlates with an increase in lipid peroxidation. Lipid peroxidation degrades the quality and structural integrity of the cell membrane by damaging polyunsaturated fatty acids. Copper is known to act as a catalyst in this process (Halliwell and Gutteridge 1985). Brown trout in OX/WS had greater peroxidation than those in OX/GC and OX/TB; and fish in OX/GC had greater peroxidation than those fish in OX/TB (Table 15). While the effects due to dietary exposure were apparent, there was no difference due to water exposure (OX/TB VS 1X/TB). Also, there was no increased lipid peroxidation in rainbow trout.

Autopsy assessment: The most apparent physical deformations observed was appearance of swollen abdomen in brown trout. Brown trout exposed to OX/WS and 1X/WS demonstrated 4% and 9% occurrence of impaction in the gut (Figure 7), whereas fish exposed to OX/TB and 1X/TB (reference diets) did not demonstrate any gut impaction. Brown trout exposed to the OX/GC treatment had a 3% occurrence of gut impaction. This condition was not observed in brown trout from the 1X/GC treatment or in rainbow trout from any of the treatments. Further evaluation of the impacted gut at day 88 reveled a swollen stomach and intestine due to excess feed material which was not passing

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through the gut.

During our daily monitoring of the experiment, we observed an unusual condition which appeared to be related to the gut impaction, but was more common. Brown trout and rainbow trout receiving the WS and GC diets were depositing fecal material in long ribbons in comparison to the shorter length and diameter fecal material present in the TB diet tanks (Figure 8). The condition of fishes receiving the WS and GC diets appeared to be one of constipation. In the worst cases, as with brown trout, this condition lead to impaction in the gut, enlargement of the stomach, and sometimes death.

DISCUSSION

The concentrations of As, Cd, Cu, Pb, and Zn measured in the TB diet in this study were similar to those measured in Turah Bridge invertebrates from the previous study (Woodward et al. 1993). However, the diets collected at WS in this study had about a 2-fold lower concentration of As, Cu, and Pb when compared to invertebrates collected from this location in the previous study. Both diets were collected from the Clark Fork River near Warm Springs and handled in an identical manner. A comparison of each metal in the WS diet from this study and the previous study was as follows: As, 19 and 40 ug/g; Cd, 0.26 and 2.2 ug/g; Cu, 174 and 390 ug/g; Pb, 15 and 27 ug/g, and Zn 648 and 1,006 ug/g. The invertebrates collected at Gold Creek for this study did not have the downstream reduction in metals concentration that was expected. While the Gold Creek station was approximately 80 km downstream from the Warm Springs station, the concentration of As, Cu, Pb, and Zn in the two diets was similar.

Decreased growth and increased tissue metals measured in brown trout from the GC and WS diet treatments in this study were similar to effects observed in the OX/CFI pvm treatment tested one year earlier (Woodward et al. 1993). The CFI pvm diet was collected at Warm Springs and handled identical to the WS diet used in this study. Mean concentrations of As, Cd, and Cu measured in tissue of rainbow trout fed the CFI pvm diet were significantly increased and growth was significantly reduced when

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compared with fish in OX water and receiving diets collected at Turah Bridge. The tissue metals present in rainbow trout after 91 days of feeding the CFI pvm diet were as follows: As, 1.5 ug/g; Cd, 0.12 ug/g; Cu, 7.8 ug/g; and Pb 0.48. Comparing these measured values with those for brown trout from the OX/WS treatment after 88 d in this study (Tables 7, 9, 11, and 13), concentrations of Cu and Cd compare closely; As accumulation was half, and Pb accumulation was 2 times higher.

One of the interfering factors in interpreting the data from the earlier study (Woodward et al. 1993) was that the energy or caloric content was greater in the control diet than the reference diet (TBI pvm VS CFI pvm). Even though we over fed both diets by 25% and fish were receiving more feed than they were consuming, we could not rule out the possibility that different energy content of test diets may have contributed to growth differences. Caloric content in this study was not a factor in comparing growth rates between the TB and WS diets. Caloric content was highest in the WS diet, but growth of both brown trout and rainbow trout on the WS diet was significantly reduced when compared to growth on the TB diet.

In the first years study, rainbow trout from all treatments fed equally well (Woodward et al. 1993). Rainbow trout in this study had reduced food intake after 50 d on the GC and WS diets which could explain the reduction in both growth and metals concentration. However, As was significantly elevated in both the tissue and the liver of all rainbow trout on the GC and WS

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diets indicating these fish accumulated As from the diet. The condition of constipation in rainbow trout from the GC and WS treatments indicates something more than reduced food intake was affecting health. Metals in natural fish-food organisms may interfere with both food acceptance and fish physiology, and the result is reduced growth and health of the fish.

To our knowledge the symptom of constipation and impaction of the gastrointestinal tract has not been reported in fish exposed to dietary metals. However, symptoms of chronic lead poisoning in mammals are slowing of nerve conduction in the peripheral nervous system and constipation (Schwartz et al. 1988, Dreisbach 1983). The mode of action of lead toxicity in mammals would indicate a similar effect on nervous tissue and the gastrointestinal tract of fishes (Scharding et al. 1973; Stephanie Ostrowski, personal communication, Veterinary Epidemiologist, Center for Disease Control, Atlanta, Georgia). Other effects in the gut included a reduction in digestive enzyme precursors (zymogen) and the sloughing of intestinal mucosal epithelial cells in brown trout. Effects on the gut epithelium was similar to the morphological and functional alterations induced in rainbow trout intestine by dietary Cd and Pb (Crespo et al. 1986). Effects on the gut were observed in both species in this study and occurred only in the treatments where metals contaminated the diets (GC and WS).

We did not observe significant reductions in survival due to any treatments in these studies. However, fishes under

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toxicological stress in the laboratory can be kept alive because stresses of the natural environment do not exist. Also, our diets were pasteurized and fortified with vitamins and minerals to standardize the test diets as much as possible; and this may reduce the toxicity of the metals in the diet as observed in our first study (Woodward et al. 1993). Pasteurization may change the nature of amino acids and any organo-metal complexes making it less toxic (Piper et al. 1982). The addition of vitamins and minerals in diets would produced fish of better health that were more capable of withstanding metals exposure than those fish on the raw diet. Whatever the case, early life stage trout in the Clark Fork River must sustain themselves on an invertebrate food source that is neither pasteurized nor has vitamins or minerals added. In our attempt to rule out causes of mortality other than metals, we altered and probably improved the natural food source.

Metal concentrations in Clark Fork forage fishes (coarse-scale sucker, redside shiner, and slimy sculpin) collected from the Warm Springs area were 6-13 times higher than the national average for various species (unpublished data, Bill Brumbaugh, U.S. Fish and Wildlife Service, Columbia, Missouri; Schmitt and Brumbaugh 1990). The mean concentrations (ug/g wet weight, n = 6) for Clark Fork fishes were as follows: As, 1.5; Cd, 0.11; Cu, 8.6; Pb, 0.71; and Zn, 49. These concentrations in Clark Fork fishes are similar to mean measured values in brown trout exposed for 88 d to 0X/WS (As, 0.74; Cd, 0.09; Cu, 6.8; and Pb, 0.85) and to 1X/WS (As, 0.79; Cd, 0.15; Cu, 8.2; and Pb, 0.78). Therefore,

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accumulation of metals by fish during aqueous and dietary exposure in the laboratory resulted in metal residues similar to that measured in field-collected fish.

There have been other investigations into metal contamination similar to the Clark Fork River, where Cu concentrations in water were low, but remained elevated in sediments, macrophytes, and benthic invertebrates. These studies, like ours, indicate diet loading is probably the most important source of Cu accumulation in fish (Dallinger and Kautzky 1985, Lanno et al. 1987). The "food-chain effect" of metals has been described as a relationship in which biomagnification is not observed and bioconcentration factors are small, but the amount of metal transferred by food can be high enough to attain biologically harmful concentrations in fish (Dallinger et al. 1987). Once in the lumen of fish, heavy metals are absorbed into gut tissue, where they are distributed to other organs such as liver, kidney, and muscle (Dallinger and Kautzky 1985). Morphological and functional alterations have been induced in trout intestine and liver by dietary metals (Crespo et al. 1986; and Lanno et al. 1987). Reduced growth of fish receiving dietary metals could be due to the energy required for binding the metals to proteins in the liver, where they are excreted through the bile (Hodson 1988), or to decreased assimilation efficiency in the gut (Brafield and Koodie 1991).

Present and past studies (Woodward et al. 1993) indicate that Clark Fork invertebrate diets are a likely cause of

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decreased survival, growth, and health in early life stage brown trout and rainbow trout. Young-of-the-year fishes in the Clark Fork River depend on a food source of macroinvertebrates, and the metals associated with this food source present a hazard to the fishery. Fish with reduced growth rates and health status could survive in laboratory experiments, but would be eliminated from natural populations where additional stresses are present. Our experiments suggest brown trout and rainbow trout populations would be reduced in the Clark Fork River and Silver Bow Creek above Milltown reservoir.

Studies comparing trout numbers and biomasses in the upper Clark Fork River and Silver Bow Creek with reference sites are supportive of our findings (unpublished report, Don Chapman Consultants, Boise, Idaho, 1992). There was a complete absence of trout from Silver Bow Creek. Reference sites contained an average of 36-fold more juvenile trout and 11-times more adult trout than did Clark Fork River sites. Reference sites were selected to be comparable with Clark Fork River sites on the basis of geology, land type, valley bottom type, and land and water uses. Adjustments were also made for habitat and flow differences. The only remaining variable between paired Clark Fork River sites and reference sites was metals contamination. Therefore, we believe the reduced standing crop of trout in the Clark Fork River and Silver Bow Creek is due in part to the chronic metals contamination of benthic invertebrates which are important as food organisms.

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CONCLUSIONS

* Exposing early life stage brown trout and rainbow trout to simulated Clark Fork River conditions of water and dietary invertebrates resulted in reduced growth and elevated tissue metals for both species.

* As, Cd, Cu, and Pb were increased in tissue of brown trout.

* As and Cd were increased in tissue; and As was also increased in liver of rainbow trout.

* Water only exposures resulted in an increase of Cd and Pb in tissue; diet only exposures resulted in an increase of As, Cd, Cu, and Pb in tissue and As in liver.

* For those brown trout on the GC and WS diets, we observed or measured an increase in constipation, gut impaction, and cell membrane damage (lipid peroxidation) and a decrease in digestive enzyme production (zymogen).

* Rainbow trout on the GC and WS diets exhibited constipation and reduced feeding activity.

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Table 1. Mean (standard deviation in parenthesis) for measured residues of metals in filtered test water at weekly intervals, n = 15, < indicates measured value was below this number which was the detection limit of the method. Simulated Clark Fork River water without metals was 0X and with metals was 1X. Nominal metal concentrations (ug/L) were as follows: Cd 1.1, Cu 12, Pb 3.2, and Zn 50.

Exposure water and tank	Cd	Cu	Pb	Zn
0X				
Tank 2	< 0.4	< 1.2	< 1.7	13 (7.9)
Tank 4	< 0.4	< 1.2	< 1.7	14 (9.7)
Tank 6	< 0.4	< 1.2	< 1.7	13 (5.9)
Tank 8	< 0.4	< 1.2	< 1.7	14 (7.3)
Tank 10	< 0.4	< 1.2	< 1.7	13 (7.4)
Tank 12	< 0.4	< 1.2	< 1.7	13 (7.6)
1X				
Tank 1	0.93 (0.19)	12 (3.0)	3.9 (1.3)	58 (7.3)
Tank 3	0.79 (0.14)	10 (1.1)	3.7 (0.98)	55 (4.7)
Tank 5	0.77 (0.17)	11 (1.4)	3.3 (0.95)	54 (5.1)
Tank 7	0.82 (0.23)	11 (2.2)	3.2 (1.2)	54 (9.9)
Tank 9	0.87 (0.13)	11 (1.6)	3.7 (1.1)	58 (9.8)
Tank 11	0.84 (0.14)	11 (1.8)	3.5 (1.1)	55 (6.5)

Table 2. Mean (standard deviation in parenthesis) for measured constituents in diets; TB = Turah Bridge, GC = Gold Creek, WS = Warm Springs, nd = non detectable; n = 2 for % protein; n = 3 for % fat, % moisture, and % ash; all others n = 4.

Diet	Constituent measured								
	%Protein	%Fat	%Moisture	%Ash	As	Cd	Cu	Pb	Zn
TB	48	16	5.9	12	6.5	nd	87	6.9	616
	(0.18)				(0.17)		(2.2)	(0.29)	(0.52)
GC	43	15	6.4	16	19	nd	178	15	650
	(0.02)				(0.15)		(5.3)	(0.48)	(6.1)
WS	44	19	8.4	12	19	0.26	174	15	648
	(0.13)				(0.30)	(0.52)	(1.9)	(0.85)	(9.1)

Table 3. Cumulative survival of brown trout through 88 d post-hatch. Invertebrate diets were collected from 3 sites on the Clark Fork River, Montana; 0X water simulates conditions in Clark Fork without metals, 1X simulates Clark Fork with metals. Comparisons are made among water and diet combinations within each time period; values are means, n = 4, standard deviations in parenthesis.

Water	Diet	% Survival		
		Day 26	Day 52	Day 88
0X	Turah Bridge	99	99	92
		(0.77)	(1.0)	(7.6)
	Gold Creek	99	99	99
		(1.1)	(1.1)	(1.1)
	Warm Springs	99	99	98
		(0.0)	(0.0)	(2.0)
1X	Turah Bridge	99	99	96
		(0.0)	(0.0)	(3.8)
	Gold Creek	99	99	92
		(0.67)	(0.67)	(6.3)
	Warm Springs	98	98	88
		(1.3)	(1.3)	(8.1)

Table 4. Cumulative survival of rainbow trout through 88 d post-hatch. Invertebrate diets were collected from 3 sites on the Clark Fork River, Montana; 0X water simulates conditions in Clark Fork without metals, 1X simulates Clark Fork with metals. Comparisons are made among water and diet combinations within each time period; values are means, n = 4, standard deviations in parenthesis.

Water	Diet	% Survival		
		Day 18	Day 52	Day 88
0X	Turah Bridge	100	98	98
		(0.67)	(2.4)	(2.4)
	Gold Creek	99	97	95
		(0.77)	(1.8)	(1.9)
	Warm Springs	98	97	92
		(1.7)	(2.8)	(6.3)
1X	Turah Bridge	99	99	98
		(2.7)	(2.7)	(4.6)
	Gold Creek	99	99	99
		(0.77)	(1.8)	(1.8)
	Warm Springs	99	97	97
		(1.3)	(2.1)	(2.1)

Table 5. Mean weight and length of brown trout through 88 d post-hatch, standard deviation in parenthesis. Invertebrate diets were collected from 3 sites on the Clark Fork River, Montana; 0X water simulates conditions in Clark Fork without metals, 1X simulates Clark Fork with metals. Comparisons are made among water and diet combinations within each day. Means with the same letter are not significantly different.

Water	Weight (mg)		
diet	Day 26	Day 52	Day 88
0X			
Turah Bridge	74 (3.3)[a]	175 (12) [a]	568 (22) [a]
Gold Creek	70 (2.5)[b]	107 (5.8)[b]	347 (9.7)[b]
Warm Springs	68 (1.5)[bc]	112 (3.2)[b]	344 (25) [b]
1X			
Turah Bridge	68 (1.9)[bc]	130 (10) [c]	421 (59) [c]
Gold Creek	67 (1.9)[bc]	94 (3.8)[d]	267 (27) [d]
Warm Springs	66 (1.0)[c]	87 (15) [d]	285 (42) [d]

Table 5.   (Continued)

Water	Length (mm)		
diet	Day 26	Day 52	Day 88
0X			
Turah Bridge	23 (0.28)	28 (0.62)[a]	40 (0.75)[a]
Gold Creek	22 (0.35)	25 (0.47)[b]	34 (0.29)[b]
Warm Springs	22 (0.25)	25 (0.28)[b]	33 (0.72)[b]
1X			
Turah Bridge	22 (0.21)	26 (0.43)[c]	36 (1.6) [c]
Gold Creek	22 (0.30)	24 (0.23)[d]	31 (0.86)[d]
Warm Springs	22 (0.22)	24 (0.88 [d]	31 (1.6) [d]

Table 6. Mean weight and length of rainbow trout through 88 d post-hatch, standard deviation in parenthesis. Invertebrate diets were collected from 3 sites on the Clark Fork River, Montana; OX water simulates conditions in Clark Fork without metals, 1X simulates Clark Fork with metals. Comparisons are made among water and diet combinations within each day. Means with the same letter are not significantly different.

Water	Weight (mg)		
diet	Day 18	Day 53	Day 88
OX			
Turah Bridge	94 (1.3)	455 (10) [a]	1,408 (56) [a]
Gold Creek	94 (3.2)	227 (14) [b]	758 (41) [b]
Warm Springs	92 (1.7)	224 (12) [b]	789 (41) [bc]
1X			
Turah Bridge	92 (2.8)	435 (16) [c]	1,374 (45) [a]
Gold Creek	92 (1.8)	225 (3.8) [b]	830 (9.1) [c]
Warm Springs	92 (1.6)	233 (14) [b]	801 (36) [bc]

Table 6.   (Continued)

Water	Length (mm)				
diet	Day 18		Day 53		Day 88

0X				
Turah Bridge	-[1]	-[1]	38 (0.41)[a]	53 (0.73)[a]
Gold Creek	-	-	30 (0.53)[b]	42 (0.75)[b]
Warm Springs	-	-	30 (0.39)[b]	42 (0.63)[b]
1X				
Turah Bridge	-	-	37 (0.27)[a]	52 (0.49)[a]
Gold Creek	-	-	30 (0.07)[b]	43 (0.13)[b]
Warm Springs	-	-	30 (0.51)[b]	42 (0.90)[b]

[1] Length data not collected on day 18 of rainbow trout study.

Table 7. Tissue copper (Cu) concentrations based on wet weight of whole brown trout on three dates and the livers of brown trout on the 3rd date. Brown trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in The Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. [a] = significantly greater than the reference treatment (0X, Turah Bridge); [b] = significantly greater than the 1X water reference diet (1X, Turah Bridge).

			Tissue Cu (µg/g)			
Water	Diet	N	Day 26	Day 52	Day 88	Day 88 - liver only
0X	Turah Bridge	4	1.63 (0.16)	1.33 (0.05)	2.44 (0.47)	22 (1.94)
0X	Gold Creek	4	1.26 (0.06)	1.96[a] (0.21)	5.26[a] (0.18)	38 (6.08)
0X	Warm Springs	4	1.52 (0.29)	1.91 (0.07)	6.80[a] (0.59)	38 (2.30)
1X	Turah Bridge	4	1.75 (0.15)	2.12[a] (0.23)	3.53 (0.25)	45 (8.92)
1X	Gold Creek	4	1.56 (0.33)	3.27[ab] (0.38)	6.88[ab] (0.80)	52 (5.19)
1X	Warm Springs	4	1.46 (0.19)	2.38[a] (0.14)	8.23[ab] (1.47)	61 (6.85)

Table 8. Tissue copper (Cu) concentrations based on wet weight of whole rainbow trout on three dates and the livers of rainbow trout on the 3rd date. Rainbow trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in The Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. ᵃ = significantly greater than the reference treatment (0X, Turah Bridge); ᵇ = significantly greater than the 1X water reference diet (1X, Turah Bridge).

			Tissue Cu (µg/g)			
Water	Diet	N	Day 18	Day 53	Day 88	Day 88 - liver only
0X	Turah Bridge	4	1.12 (0.04)	1.86 (0.05)	3.08 (0.14)	36 (1.9)
0X	Gold Creek	4	1.13 (0.03)	2.79ᵃ (0.07)	3.19 (0.21)	21 (2.1)
0X	Warm Springs	4	1.21 (0.10)	3.22ᵃ (0.23)	4.27 (0.95)	27 (3.4)
1X	Turah Bridge	4	1.44 (0.16)	1.90 (0.20)	2.89 (0.06)	41 (2.3)
1X	Gold Creek	4	1.39 (0.06)	3.68ᵃᵇ (0.47)	3.38 (0.24)	23 (0.5)
1X	Warm Springs	4	1.66ᵃ (0.25)	2.27ᵃᵇ (0.26)	4.01 (0.77)	30 (2.7)

Table 9. Tissue arsenic (As) concentrations based on wet weight of whole brown trout on three dates and the livers of brown trout on the 3rd date. Brown trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in The Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. [a] = significantly greater than reference treatment (0X, Turah Bridge); [b] = significantly greater than the 1X water reference diet (1X, Turah Bridge).

Tissue As (µg/g)

Water	Diet	N	Day 26	Day 52	Day 88	Day 88 - liver only
0X	Turah Bridge	4	0.16 (0.04)	0.08 (0.03)	0.19 (0.03)	0.12 (0.10)
0X	Gold Creek	4	0.37 (0.03)	0.24[a] (0.04)	0.66[a] (0.02)	0.14 (0.07)
0X	Warm Springs	4	0.39 (0.03)	0.32[a] (0.03)	0.74[a] (0.05)	0.07 (0.07)
1X	Turah Bridge	4	0.18 (0.03)	0.10 (0.01)	0.18 (0.01)	0.10 (0.12)
1X	Gold Creek	4	0.43 (0.05)	0.30[ab] (0.03)	0.71[ab] (0.05)	0.38 (0.29)
1X	Warm Springs	4	0.30 (0.04)	0.23[ab] (0.07)	0.79[ab] (0.10)	0.39 (0.25)

Table 10. Tissue arsenic (As) concentrations based on wet weight of whole rainbow trout on three dates and the livers of rainbow trout on the 3rd date. Rainbow trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; OX water simulates conditions in the Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. [a] = significantly greater than the reference treatment (OX, Turah Bridge); [b] = significantly greater than the 1X water reference diet (1X, Turah Bridge).

			Tissue As (µg/g)			
Water	Diet	N	Day 18	Day 53	Day 88	Day 88 - liver only
OX	Turah Bridge	4	0.03 (0.04)	0.19 (0.03)	0.28 (0.01)	0.24 (0.10)
OX	Gold Creek	4	0.09 (0.02)	0.57[a] (0.02)	0.58[a] (0.03)	0.71[a] (0.06)
OX	Warm Springs	4	-0.02 (0.06)	0.64[a] (0.02)	0.72[a] (0.07)	0.88[a] (0.07)
1X	Turah Bridge	4	-0.01 (0.07)	0.20 (0.04)	0.22 (0.02)	0.28 (0.01)
1X	Gold Creek	4	-0.02 (0.09)	0.51[ab] (0.10)	0.58[ab] (0.11)	0.82[ab] (0.03)
1X	Warm Springs	4	0.04 (0.06)	0.56[ab] (0.11)	0.63[ab] (0.13)	0.88[ab] (0.03)

Table 11. Tissue lead (Pb) concentrations based on wet weight of whole brown trout on three dates and the livers of brown trout on the 3rd date. Brown trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in The Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. [a] = significantly greater than the reference treatment (0X, Turah Bridge); [b] = significantly greater than the 1X water reference diet (1X, Turah Bridge).

Water	Diet	N	Tissue Pb (µg/g)			
			Day 26	Day 52	Day 88	Day 88 - liver only
0X	Turah Bridge	4	0.19 (0.01)	0.27 (0.09)	0.19 (0.11)	0.65 (0.14)
0X	Gold Creek	4	0.22 (0.06)	0.24 (0.04)	0.48[a] (0.12)	4.99 (2.39)
0X	Warm Springs	4	0.17 (0.01)	0.21 (0.01)	0.85[a] (0.10)	1.83 (0.93)
1X	Turah Bridge	4	0.19 (0.04)	0.27 (0.05)	0.52[a] (0.16)	1.34 (0.38)
1X	Gold Creek	4	0.49 (0.31)	1.10 (0.58)	0.88[ab] (0.08)	2.83 (0.75)
1X	Warm Springs	4	0.20 (0.02)	0.39 (0.05)	0.78[ab] (0.10)	1.97 (0.26)

Table 12. Tissue lead (Pb) concentrations based on wet weight of whole rainbow trout on three dates and the livers of rainbow trout on the 3rd date. Rainbow trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in the Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. a = significantly greater than the reference treatment (0X, Turah Bridge).

			Tissue Pb (µg/g)			
Water	Diet	N	Day 18	Day 53	Day 88	Day 88 - liver only
0X	Turah Bridge	4	0.14 (0.04)	0.28 (0.19)	0.23 (0.08)	0.24 (0.04)
0X	Gold Creek	4	0.14 (0.01)	0.29 (0.07)	0.19 (0.07)	0.39 (0.04)
0X	Warm Springs	4	0.14 (0.01)	0.24 (0.04)	0.25 (0.10)	0.15 (0.05)
1X	Turah Bridge	4	0.15 (0.03)	0.23 (0.02)	0.39 (0.06)	0.46 (0.20)
1X	Gold Creek	4	0.21 (0.04)	0.33 (0.12)	0.21 (0.06)	0.47 (0.13)
1X	Warm Springs	4	0.15 (0.01)	0.35 (0.05)	0.46 (0.06)	0.18 (0.07)

Table 13. Tissue cadmium (Cd) concentrations based on wet weight of whole brown trout on three dates and the livers of brown trout on the 3rd date. Brown trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; OX water simulates conditions in The Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. [a] = significantly greater than reference treatment (OX, Turah Bridge); [b] = significantly greater than the 1X water reference diet (1X, Turah Bridge).

			Tissue Cd (μg/g)			
Water	Diet	N	Day 26	Day 52	Day 88	Day 88 - liver only
OX	Turah Bridge	4	0.015 (0.003)	-0.015 (0.039)	0.035 (0.006)	0.09 (0.04)
OX	Gold Creek	4	0.003 (0.005)	0.013 (0.010)	0.048 (0.003)	0.20 (0.09)
OX	Warm Springs	4	0.008 (0.006)	0.025 (0.010)	0.090[a] (0.016)	0.17 (0.15)
1X	Turah Bridge	4	0.033 (0.010)	0.073[a] (0.005)	0.090[a] (0.014)	0.39 (0.14)
1X	Gold Creek	4	0.035 (0.003)	0.133[ab] (0.020)	0.130[ab] (0.007)	0.44 (0.07)
1X	Warm Springs	4	0.045[a] (0.009)	0.120[a] (0.015)	0.145[ab] (0.012)	0.37 (0.07)

Table 14. Tissue cadmium (Cd) concentrations based on wet weight of whole rainbow trout on three dates and the livers of rainbow trout on the 3rd date. Rainbow trout were fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in the Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Standard error of the mean is in parentheses. [a] = significantly greater than the reference treatment (0X, Turah Bridge).

			Tissue Cd (µg/g)			
Water	Diet	N	Day 18	Day 53	Day 88	Day 88 - liver only
0X	Turah Bridge	4	-0.08 (0.04)	0.010 (0.015)	0.05 (0.01)	0.10 (0.01)
0X	Gold Creek	4	-0.02 (0.03)	0.025 (0.011)	0.04 (0.01)	0.06 (0.02)
0X	Warm Springs	4	-0.11 (0.04)	0.010 (0.004)	0.04 (0.01)	0.08 (0.02)
1X	Turah Bridge	4	-0.07 (0.02)	0.068 (0.018)	0.14[a] (0.01)	0.24[a] (0.01)
1X	Gold Creek	4	-0.08 (0.07)	-0.010 (0.100)	0.10[a] (0.01)	0.07 (0.02)
1X	Warm Springs	4	-0.06 (0.05)	0.002 (0.103)	0.16[a] (0.02)	0.16 (0.02)

Table 15. Mean lipid peroxidation (standard error in parentheses) of brown and rainbow trout fed invertebrate diets collected from three sites on the Clark Fork River, Montana; 0X water simulates conditions in The Clark Fork River without metals, 1X simulates the Clark Fork River with metals. Lipid peroxidation value is expressed as the relative intensity of a flourometric measurement of a chloroform extract of whole fish tissue collected at the end of the study. Means with the same letter are not significantly different.

Water and diet	N	Brown trout	Rainbow trout
0X			
Turah Bridge	4	1.68 (0.15)^a	1.39 (0.15)
Gold Creek	4	2.52 (0.06)^{ab}	1.22 (0.10)
Warm Springs	4	3.99 (0.27)^c	1.52 (0.40)
1X			
Turah Bridge	4	1.80 (0.09)^a	0.94 (0.07)
Gold Creek	4	2.84 (0.45)^{ab}	1.57 (0.23)
Warm Springs	4	3.85 (0.63)^{bc}	1.68 (0.51)
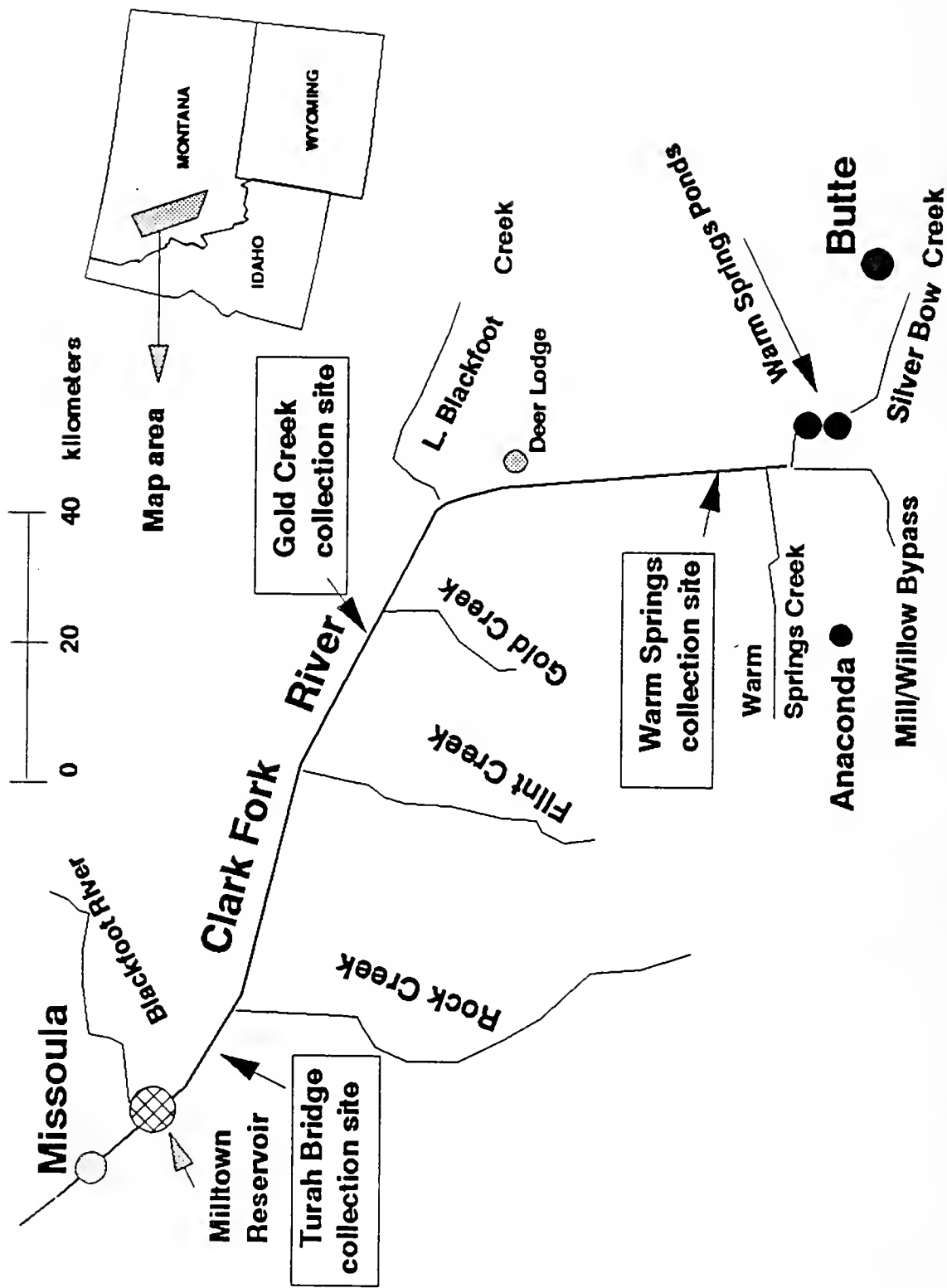
Figure 1. Upper Clark Fork River from confluence of Silver Bow Creek, Warm Springs Creek, and Mill/Willow Creek to Missoula, Montana. Aquatic invertebrates were collected at the sites indicated and used as representative food-chain organisms in the diet.
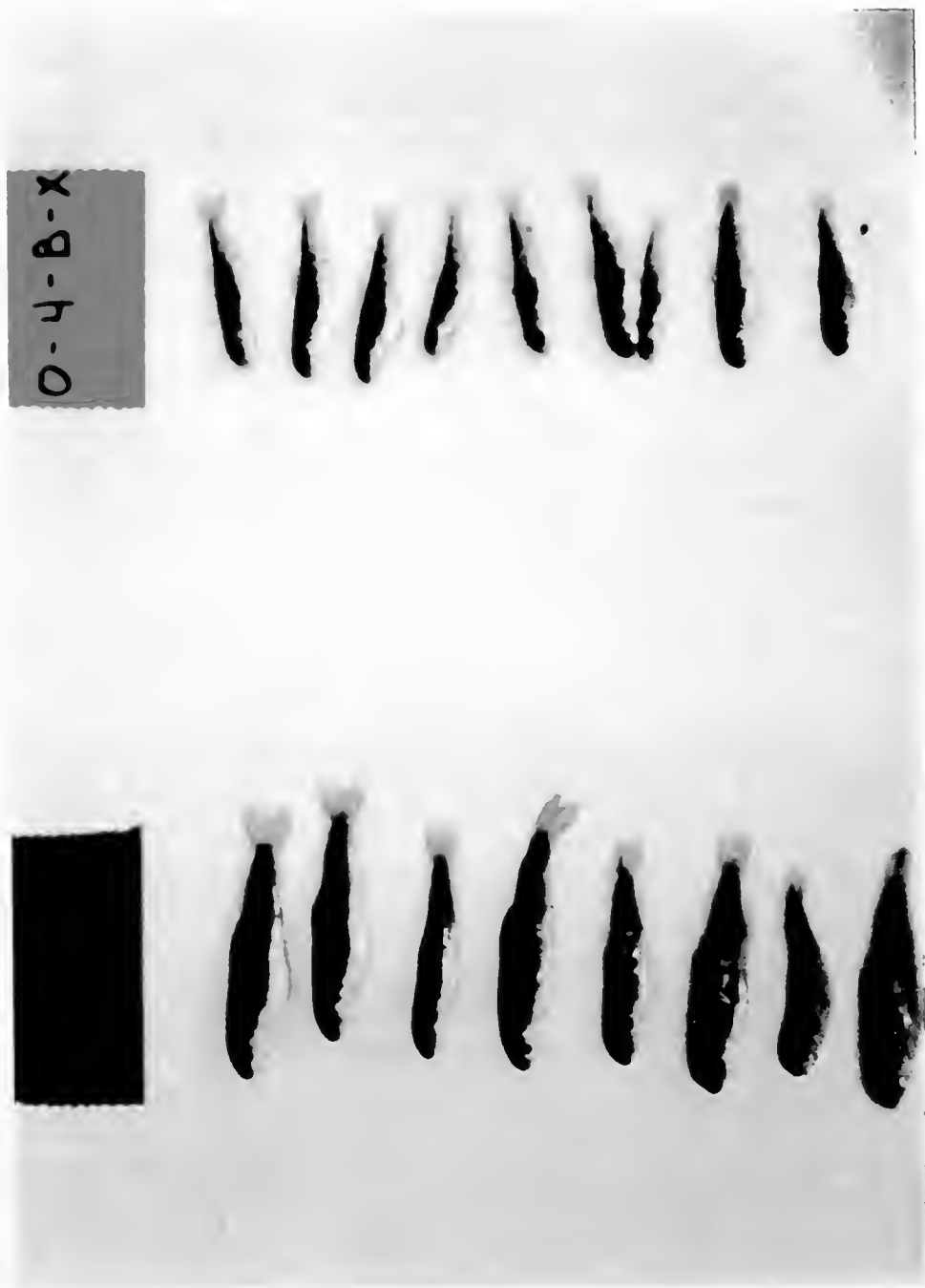
Figure 2.   Visible differences in rainbow trout growth to 88 d in 0X test water when fed Warm Springs invertebrates (right column) versus Turah Bridge invertebrates (left column).
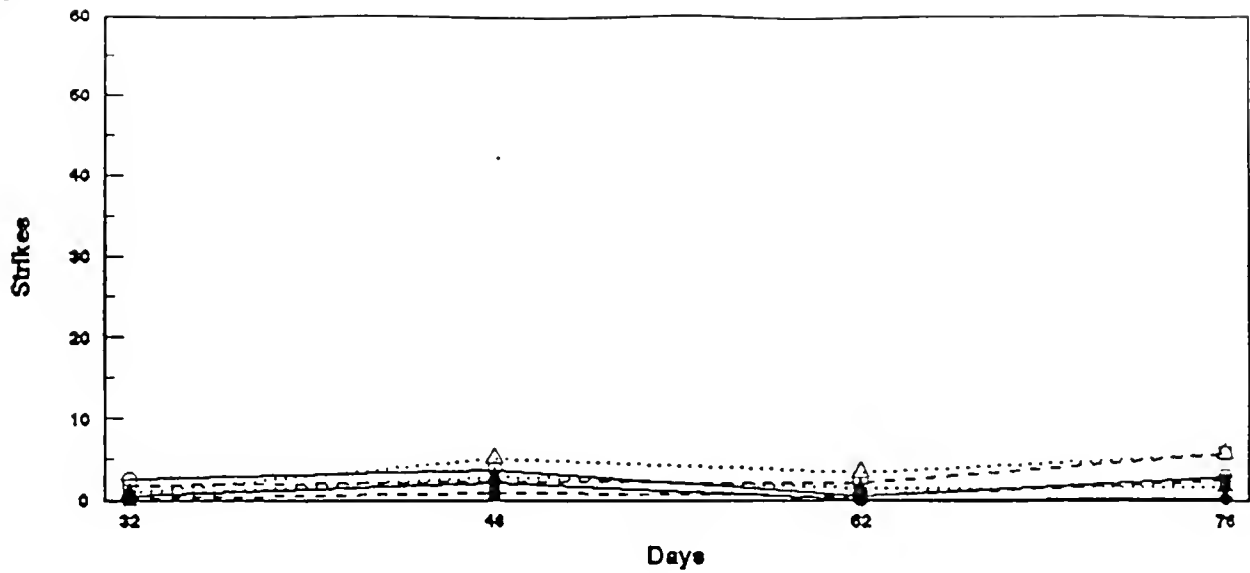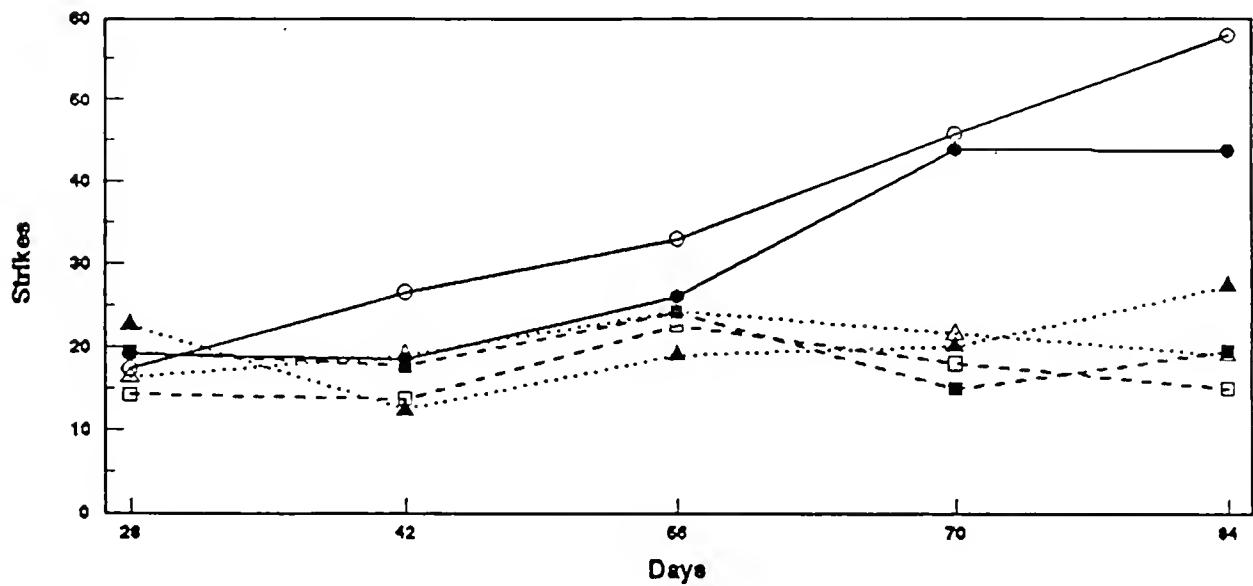
Figure 3. Mean feeding activity of brown trout (A) and rainbow trout (B).
Observations are the number of strikes directed at food during a 2 min
observation period. Treatment codes are as follows:

OX Turah Bridge	OX Gold Creek	OX Warm Springs
—○—	- ☐ - ·	· · △ · ·
1X Turah Bridge	1X Gold Creek	1X Warm Springs
—●—	- ■ - ·	· · ▲ · ·
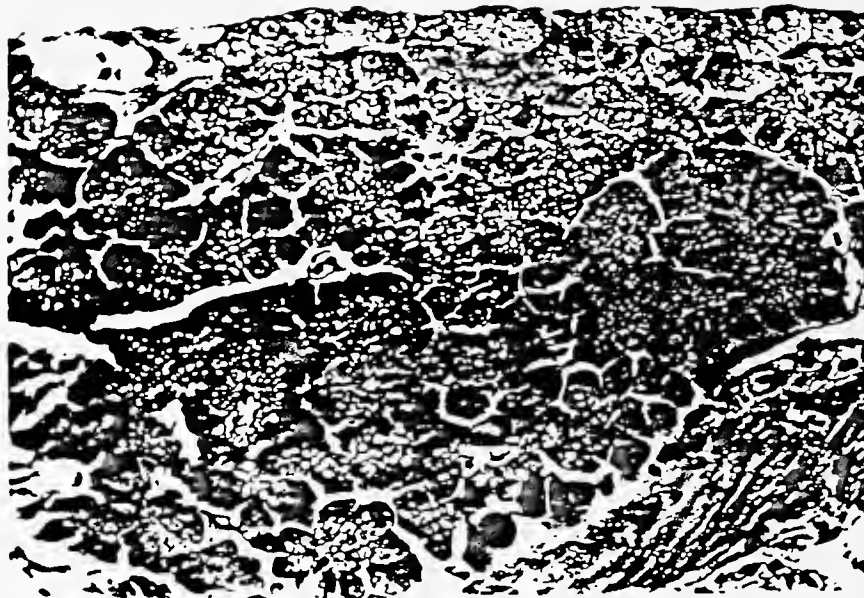
Figure 4. Normal pancreatic cells showing abundant zymogen granules in cytoplasm of cells (0X/TB diet). X450

Figure 5. Pancreatic tissue devoid of zymogen granules (0X/WS diet). X450

Figure 6.   Note vacuolation and degeneration of intestinal, mucosal-epithelial cells (OX/WS diet). X450

Figure 7. Impacted gut observed in brown trout fed the Warm Springs Invertebrate diet (fish on left).

Figure 8. Larger feces size of brown trout fed Warm Springs diet (right beaker as compared to feces of same age fish on Turah Bridge diet (left beaker).

AQUATIC RESOURCES INJURY REPORT

APPENDIX F

The Physiological Impairment of Fish Caused by Chronic Exposure to Metals at Concentrations Typically Found in Clark Fork River Food and Water

Prepared by:

Harold Bergman, University of Wyoming

FINAL REPORT

The physiological impairment of fish caused by chronic exposure to metals at concentrations typically found in Clark Fork River food and water.

Research Report on Injury Determination

Fishery Protocol #2

Assessment Plan, Part I

Clark Fork River Basin NPL Sites, Montana

Submitted by:

Harold L. Bergman

Red Buttes Environmental Biology Laboratory

University of Wyoming

Laramie, Wyoming 82071

May, 1993

INTRODUCTION

Surface water and sediments from the upper Clark Fork River contain elevated concentrations of metals including As, Cd, Cu, Pb and Zn (USGS 1989, Lambing 1991, Moore et al. 1991). However, measurements of pollutants in water and sediment alone are sometimes difficult to link to fish health because fish move in and out of polluted areas and because water quality fluctuates through time (Flemming and Trevors 1989). Additionally, water and sediment analyses do not account for toxicity caused by ingestion of contaminated food (Komarovsky and Polishchuk 1981). Physiological responses (pathology) caused by exposure to metals and accumulation of metals in tissues can be used to supplement fish population and water and sediment quality monitoring and help in determining cause and effect relationships.

The objectives of this study were to determine the degree of fish health impairment caused by chronic exposure of trout to metals in the Clark Fork River and in laboratory experiments simulating metal exposures in food and water typical of the Clark Fork River. Adult brown trout were collected from the Clark Fork River and from two reference sites for metal residue determination and determination of physiological parameters related to fish health. Additionally, metal residues and physiological parameters related to fish health were determined in brown trout and rainbow trout swim-up fry sampled from a companion laboratory study (see Appendix E to the Aquatic Resource Injury Assessment Report, Woodward et al. 1993a), where fish were exposed for 88 days to metal concentrations in water and food typical of the Clark Fork River.

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METHODS

Procedures employed for this study were those specified under Research Plans, Section 7.4.4, in the Assessment Plan: Part I, Clark Fork River NPL Sites, Montana (Montana 1992) except where noted.

Sample Collection, Field

During May- 13-14, 1992 adult brown trout were collected by electrofishing from each of four sites (Figure 1). Two of the sites were located on the Clark Fork River, an upstream site below Warm Springs ponds and a downstream site near Turah Bridge. These sites include "high" and "low," respectively, measured concentrations of metals in water and sediments. The third and fourth sites were reference sites located on the Big Hole River and Rock Creek (a tributary to the Clark Fork River). A total of ten fish were collected at the Warm Springs and Big Hole River sites; nine fish were collected at the Turah Bridge and Rock Creek sites. [NOTE: A second control site was included in the sampling design instead of the Gold Creek site as stated in the assessment plan.]

In the field, each fish was measured for length and weight, examined with an autopsy assessment procedure (Goede 1989) and sampled for blood, gill, liver, kidney, pyloric caeca and large intestine. Sections of each tissue (and the whole spleen) from each fish were placed in Bouin's solution for subsequent histological examination. The remaining portion of tissue was frozen immediately in liquid nitrogen and placed on dry ice for transport to the University of Wyoming Red Buttes Environmental Biology Laboratory where they were stored at -70°C. Each tissue was later ground in a liquid nitrogen cooled mortar and pestle and divided into aliquots for tissue metal, lipid peroxidation, metallothionein and stress protein measurements. [NOTE: The

samples were divided into aliquots for residue and physiological measurements in the laboratory rather than in the field.] Five additional fish were collected and frozen for whole body residue analyses. [NOTE: Fish for whole body analyses were not collected from Rock Creek and water samples were not collected from any of the sites.]

Sample Collection, Laboratory

Brown trout and rainbow trout were exposed to metals in the water and fed invertebrate diets from the Clark Fork River. Details of these experiments are presented in Appendix E to the Aquatic Resource Injury Assessment Report (Woodward et al. 1993a). Whole fish were collected on two dates during the experiments and at the termination of the experiments for analyses of tissue metal residues. Autopsy assessments were performed and whole fish were collected at the termination of the experiments for histopathology, lipid peroxidation and metallothionein analyses. Liver and gill samples were collected at the termination of the experiments for stress protein analyses. All samples except for histopathology (which were placed in Bouin's solution) were frozen immediately and transported to the University of Wyoming Red Buttes Environmental Biology Laboratory where they were stored at -70°C.

Metal Residues in Fish Tissues

Fish tissues were lyophilized, digested with nitric acid in a microwave, and taken up to a 50 ml final volume in a 5% HNO_3 matrix at the Department of Health and Environmental Science, Chemistry Laboratory Bureau, Helena, Montana. The samples were then transported to Butte, Montana for chemical analyses by the Montana Bureau of Mines Analytical Laboratory. Fish tissues were analyzed for As, Cu, Cd, and Pb using inductively coupled plasma emission - mass spectroscopy (ICP-MS) on a Perkin Elmer Elan 5000 [NOTE: Tissue Zn was not measured]. For analyses the samples were diluted and an internal standard solution containing Y,

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In, and Bi was added to each sample at the time of dilution to a concentration of 50 μg/L for each of the internal standards.

Analytical protocols defined by EPA Method 200.8 were followed with minor deviations. In setting up the instrument for analysis, the recommendations of the manufacturer were followed. Initial calibration verification, continuing calibration verification, low level standard verification, and spike and duplicate measurements were used to verify the analytical method. Analytical duplicates and spikes were run every ten samples. The spikes were prepared by adding 1 ml of spike concentrate to 9 ml of the diluted digestate to give a final spike addition of 100μg/L. No appropriate reference samples were commercially available, hence we submitted duplicate tissues on a regular basis and used them for verification.

Whole fish were homogenized in a stainless steel blender, and 2-3 g samples of the homogenate were lyophilized, digested in 5 mls of 30% HNO_3, and analyzed by graphite furnace atomic absorption spectroscopy (Perkin Elmer Model 2380). Quality assurance and quality control was performed as above but internal standards containing Y, In, and Bi were not measured. [NOTE: Whole body Ca, Na and Zn were not measured.]

Autopsy Assessment

Length (mm) and weight (g) were recorded on each fish and the condition factor (KTL) was calculated as (Weight x 10^5)/Length^3. We examined the fish internally and externally for gross abnormalities using the autopsy assessment method of Goede (1989). For example, opaque eyes, pale skin, eroded fins, and color and size of organs were noted. This method does not evaluate cause of abnormalities but is an index that has been used to gauge the relative health of hatchery fish.

Histology

Tissues collected and examined included gill, kidney, liver, spleen and pyloric caeca. Adipose and pancreatic tissue adjacent to pyloric caeca were also examined. All tissues were fixed in Bouin's in the laboratory or field, transported to the U.S. FWS Fish Technology Center in Bozeman, Montana where tissues were processed by standard procedures for light microscopy. Sections were cut at 4 μm, stained with hematoxylin and eosin (H&E) for morphology or rhodanine for copper, and examined by light microscopy.

Lipid Peroxidation

A fluorometric assay (Dillard and Tappel 1984, Fletcher et al. 1973) was used to measure products of lipid peroxidation. A chloroform-methanol extraction of tissue preceded the fluorometric measurement. Two hundred mg of ground sample was placed in a glass homogenizer and a 2:1 mixture of HPLC grade chloroform:methanol was added (7.00 mls for a 200 mg sample). The tissue was processed four times in a homogenizer, diluted with an equal volume of water, and homogenized four additional times. The mixture was then vortexed for 2 min and transferred to a corex tube.

The mixture was spun at 3,000 rpm for 1 min and the chloroform layer was removed. Fluorescence was measured (Aminco-Bowman SPF) at a wavelength of 425 nm emission during excitation at 340 and 360 nm. Previous measurements demonstrated that these wavelengths provided the greatest sensitivity and reliability and two excitation wavelengths were used to ensure that the measurements were reproducible. The fluorometer transmits the excitation wavelength to the sample causing it to fluoresce. Fluorescence intensity at the emission wavelength is then measured as relative intensity on the photomultiplier.

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Metallothionein

Metallothionein (MTN) was measured (in μg/g) on whole fish from the laboratory experiments and liver samples from the field. A radioimmunoassay (RIA) developed by Högstrand and Haux (1990) was used to measure MTN in ground fish tissues. This is a competitive double-antibody RIA which uses ^{125}I-perch MTN (which reacts with brown and rainbow trout MTN) as a tracer. In this assay, native MTN competes with labeled MTN for binding sites on the anti-perch MTN antibodies. Thus, as the native MTN increases, the ^{125}I measurements decrease.

Serum ions

Blood sampled from the fish in the field was thawed and spun at 300 rpm for 5 min. The serum was collected and Ca, K and Na were measured by flame atomic absorption spectrophotometry (Perkin Elmer model 2830). Replicates, duplicates and internal standards were used to verify the method.

Stress Protein

Because this assay is labor intensive, the tissues were screened by randomly choosing five samples of gill and liver collected from the Rock Creek and Warm Springs sites only. Gill and liver tissues from the control laboratory fish (Turah Bridge diet/0X water) and the fish in the most contaminated exposure (Warm Springs diet/1X water) were analyzed first for stress proteins. A t-test revealed a significant difference in gill tissues of fish from these two exposures and gill tissues from the remaining two exposures were analyzed.

Specifically, HSP 70 (a 70,000 kD protein) was the class of stress proteins measured. A protein marker made by exposing gill and liver tissues to heat shock was run alongside the samples on polyacrylamide gels. Equal amounts of protein were loaded onto each lane of the

gels. We prepared western blots (on 0.2 μm pore size PVDF paper) and incubated them with

HSP 70 monoclonal antibody (STRESS GEN). The second antibody was goat anti-mouse

conjugated with alkaline phosphatase. The alkaline phosphatase substrate was used to visualize

the reaction. The lanes of each blot were then measured with a densitometer, and stress protein

is reported as a per cent of the HSP 70 protein marker.

Statistics

All data from the laboratory experiments were analyzed with a 2-way ANOVA with diet

and water as variables followed by least squares means comparisons (SAS 1985). For the field

data, medians of the tissue metal concentrations were compared with the Wilcoxon rank sum test

(Zar 1984). Using the TOXSTAT software package (Gulley et al. 1991), metallothionein, lipid

peroxidation and serum ion measurements were analyzed by a 1-way ANOVA using sample site

as the variable. All ANOVA tests used the Tukey multiple comparison/contrast procedure to

compare means. All tests used a statistical significance of 0.05 ($\alpha = 0.05$) and data was

transformed when necessary to meet the normality and homogeneity assumptions of an ANOVA.

Preliminary stress protein measurements were analyzed with a t-test at $\alpha = 0.05$, if a significant

difference was observed, additional samples were measured and a 1-way ANOVA followed by a

Tukey means comparison was performed. For statistical analyses of data from the field samples,

Rock Creek and Big Hole values were pooled to establish a baseline value (Combined

Reference). To calculate the means, all values less than the detection limit were recorded as the

detection limit concentration. However, all of the original data was used to calculate the standard

errors of the means (Gilbert 1987, Porter et al. 1988).

RESULTS

Metal Residues in Tissues, Laboratory

In all cases, brown trout accumulated higher residues of whole body metals than rainbow trout. This may have resulted from the reduced feeding observed in rainbow trout. Brown trout accumulated significant residues of Cu, As, Cd and Pb via diets collected from the Clark Fork River near Gold Creek and below Warm Springs Ponds. In addition, brown trout accumulated significant residues of Cd and Pb via the water exposure. Rainbow trout accumulated significant residues of As in both whole body and liver tissues from the Gold Creek and Warm Springs diets. And whole body Pb increased in rainbow trout exposed to metals in the water. For more detail on residue accumulation in the laboratory feeding study see Appendix E to the Aquatic Resource Injury Assessment Report (Woodward et al. 1993a).

Metal Residues in Tissues, Field

We observed significantly elevated concentrations of Cu, As and Cd in liver, kidney and pyloric caeca of adult brown trout collected from Warm Springs as compared to the reference sites. In addition, Cu and Cd were significantly elevated in gill, and Cu was also significantly elevated in whole body samples. What follows is a more detailed description of metal concentrations observed. The metal residues are presented for each of the four analyzed metals in Figures 2, 3, 4, and 5, and the mean values and standard errors of the means (SEM) for metal residues are also presented by tissue type in Appendix Tables 1-5 (as μg metal/g dry wt.) and Appendix Tables 6-9 (as μg metal/g wet wt.). In the results presented in Figures 2-5 and in the Appendix Tables, mean values $\pm$ SEM are shown to represent the traditional comparator statistics (means) along with a demonstration of variance (SEMs). However, the results of actual statistical comparisons presented in the Figures and Appendix Tables are from the non-parametric

Wilcoxon rank sum test for medians (see Methods section). In these statistical comparisons of median metal residues for individual tissues by site, the comparisons are between reference values (the Combined Reference which consists of measurements from fish collected from both Rock Creek and Big Hole) and the Clark Fork River sites.

Copper: All tissues measured in fish from Warm Springs had significantly elevated concentrations of Cu when compared to the reference sites (Figure 2 and Appendix Tables 1-5). Liver and whole body samples collected from Turah Bridge also had elevated concentrations of Cu when compared to the reference sites. The greatest magnitude of elevation in tissue Cu concentrations were observed in the liver. Livers of fish from below Warm Springs ponds had 2394 μg Cu/g dry weight while livers from the reference sites had 759 μg Cu/g dry weight.

Arsenic: Arsenic concentrations in the liver, kidney and pyloric caeca of brown trout collected from below Warm Springs were significantly elevated above the reference sites (Figure 3 and Appendix Tables 1-4). The kidney accumulated the highest residues of As; kidneys of fish from below Warm Springs had 11.29 μg As/g dry weight compared to 1.83 μg As/g dry weight from the combined reference. Kidneys of fish from Turah Bridge also had significantly higher residues of As than fish from the combined reference. Arsenic in the gill and liver of fish from Turah Bridge were significantly lower than the reference sites. [NOTE: Arsenic was not measured in the whole fish samples.]

Cadmium: Cadmium concentrations were significantly elevated in gill, liver, kidney and pyloric caeca of brown trout from below Warm Springs compared to the reference sites (Figure 4 and Appendix Tables 1-5). Concentrations of Cd in the kidney and pyloric caeca of fish from Turah Bridge were also significantly higher than the reference sites. The greatest concentrations of Cd were observed in the kidney, as fish from near Turah Bridge and below Warm Springs had

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mean concentrations of 4.39 and 8.56 μg Cd/g dry weight, respectively, compared to 1.33 μg Cd/g dry weight observed in fish from the combined reference. There were no significant differences in whole body Cd concentrations among sites.

Lead: The concentrations of Pb in the kidneys of fish from near Turah Bridge and below Warm Springs were significantly higher than in the kidneys of fish collected at the reference sites (Figure 5 and Appendix Table 1-5). However, the Pb concentration in pyloric caeca of fish from the reference is significantly greater than from fish collected near Turah Bridge. We measured a very high concentration of Pb (39 μg/g dry weight) in <u>one</u> pyloric caeca sample collected from Rock Creek; it is likely that this sample was contaminated with Pb. The Wilcoxon rank sum test did not detect a significant difference between pyloric caeca of fish from the reference and those from below Warm Springs in spite of a marked difference in the mean values (the median value is not markedly affected by the one extremely high value, while the mean value is affected). There were no significant differences in gill or whole body Pb concentrations among sites.

Autopsy Assessment

Impacted guts were observed in brown trout exposed to water and dietary metals in the laboratory. The autopsy assessment at the termination of these experiments confirmed that the guts of brown trout fed a diet collected from below Warm Springs were severely impacted. In addition, other organs in fish with gut impaction appeared smaller than normal. No other abnormalities were observed during the autopsy assessment of fish from the laboratory exposures. For more detailed results of the autopsy assessment from the laboratory experiments see Appendix E to the Aquatic Resource Injury Assessment Report (Woodward et al. 1993a).

We noted few differences in parameters measured during the autopsy assessment of adult brown trout collected in the field. Differences were not observed in the condition of the eye,

gill, pseudobranch, hind gut, kidney, liver, fin and opercula among fish collected from the four sites (Appendix Table 10). The most notable difference found during the autopsy assessment was that the KTL was reduced in brown trout collected from below Warm Springs (0.31) compared to the combined reference (0.69), but this difference is not significant (Table 1). There were no significant differences in weights or lengths of fish among sites.

Histology

Brown trout fed a diet collected from below Warms Springs had a decreased number of zymogen granules in their pancreatic cells. Because zymogen granules are precursors of digestive enzymes, a decrease in their number will have detrimental effects on the digestive capability of the affected fish. Swelling and vacuolar degeneration of pancreatic tissue and severe vacuolation and sloughing of intestinal mucosal epithelial cells were also observed in fish fed a diet collected from below Warm Springs (see Appendix E to the Aquatic Resource Injury Assessment Report, Woodward et al. 1993a, for additional information).

Severe lesions were not observed in any of the tissues examined from adult brown trout collected from the field. However, groups of fish were distinguishable based on pathological changes. Livers of fish collected from the Warm Springs, Turah Bridge and Rock Creek sites (but not the Big Hole River site) contained variable amounts of copper inclusions within the cytoplasm of hepatocytes.

Most of the cellular changes observed in the four groups of fish are commonly seen in older fish. Numerous spleen hematomas, degeneration of individual hepatocytes, abundant melanomacrophages, and sclerosis of renal glomeruli were noted in all groups of fish examined.

Liver sections from one of eight fish collected from Rock Creek was lightly positive for Cu (few focal areas within hepatocytes containing small granules that stained positive for Cu).

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Liver sections of two of nine fish from Turah Bridge: mild (1), moderate (1), stained positive for Cu and liver sections from eight of the ten fish collected from Warm Springs contained intracytoplasmic granules that stained positive for copper: mild (4), moderate (1), extensive (3). In addition, nuclear vacuolation of hepatocytes was noted in three fish collected from Rock Creek, seven fish collected from Turah Bridge and four fish collected from Warm Springs.

Lipid Peroxidation

Brown trout fed a diet collected from below Warm Springs had a significant increase in lipid peroxidation (measured as an increase in relative intensity). Lipid peroxidation was measured on whole fish and is probably the result of peroxidation in many different tissues. There was no increase in products of lipid peroxidation in rainbow trout (see Appendix E to the Aquatic Resource Injury Assessment Report, Woodward et al. 1993a, for additional information).

Differences in lipid peroxidation were observed among the brown trout from the different field sites for large intestine, liver and pyloric caeca (Table 2). Lipid peroxidation was significantly greater in large intestine, liver and pyloric caeca of fish collected from below Warm Springs than the same tissues of fish from Turah Bridge and the reference sites.

Metallothionein

There was no significant increase in metallothionein concentrations in brown or rainbow trout fed a diet collected from below Warm Springs. The small size of these fish necessitated analysis of whole fish. Because metallothionein is predominantly present in the liver and kidney (Olsson and Högstrand 1987), any differences may have been masked by the whole tissue determinations (see Appendix E to the Aquatic Resource Injury Assessment Report, Woodward et al. 1993a, for additional information).

Livers of fish from below Warm Springs had a significantly greater concentration of

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metallothionein than the livers of fish from Turah Bridge or the reference sites (Table 3). The concentration of metallothionein in the livers of fish from below Warm Springs was more than twice the concentration measured in fish from the reference sites.

Serum Ions

The concentration of serum Na was significantly lower in fish from Turah Bridge than in fish from the combined reference and in fish from below Warm Springs (Table 4). The concentration of serum Ca was significantly lower in fish from Warm Springs than in fish from Turah Bridge, and there were no significant differences in serum K from fish among sites.

Stress Protein

The stress protein results are difficult to interpret. Because the antibody was prepared for reactions with mouse rather than fish tissue, the antibody reaction was minimal and although we used the highest quality PVDF paper for western blots, imperfections in the paper (and possibly other undetected proteins) interfered with the densitometer measurements. For this reason, the placement of the baseline during the densitometer readings was subjective. Also, on occasion we observed a cross-reaction of the HSP 70 anti-body with a lower molecular weight product. It is unknown at this time if these were breakdown products of HSP 70 or another HSP altogether. These problems make us question the quantitative use of the stress protein results presented in Table 5 and in Appendix E to the Aquatic Resource Injury Assessment Report (Woodward et al. 1993a).

DISCUSSION

Metal Residue Accumulation

Residues of Cu, As and Cd were significantly elevated in tissues of adult brown trout collected in May 1992 from the Clark Fork River as compared to reference sites in Rock Creek and the Big Hole River. Residues were particularly high in fish from below Warm Springs Ponds, where Cu and Cd were significantly elevated in all tissues analyzed (gill, liver, kidney and pyloric caeca -- Figures 2 and 4), and where As residues were significantly elevated in three of the four tissues analyzed (liver, kidney and pyloric caeca -- Figure 3). Tissue samples from brown trout collected near Turah bridge were also significantly elevated over the reference sites for Cu in liver, As in kidney, and Cd in kidney and pyloric caeca (Figures 2, 3 and 4).

Copper was the most highly elevated of the four metals analyzed (Figures 2, 3, 4 and 5). Mean Cu residue concentrations (dry weight basis) in liver were 2394 μg/g at Warm Springs, 1079 μg/g at Turah Bridge and 759 μg/g at the combined reference sites (Figure 2). Also, of the metals analyzed, Cu was the only metal with significantly elevated whole body residues, with Warm Springs fish (6.36 μg/g dry wt.) significantly higher than Turah Bridge fish (4.26 μg/g dry wt.), which were significantly higher than fish from the reference site (Big Hole, 3.04 μg/g dry wt.).

Whole body Cu residues reported here for Clark Fork brown trout (Figure 2 and Table 6) were lower than those reported as early as 1974 for fish collected from the same reaches of the river. For instance, the Anaconda Company analyzed brown trout and whitefish in 1974 and reported whole body Cu concentrations (μg/g dry wt.) of 7.2 below Warm Springs, 7.2 near Racetrack, 7.0 near Rock Creek and 4.5 below Drummond (Dent 1974). Results of more recent analyses of Cu residues in Clark Fork River brown trout tissues are very similar to liver, gill and

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kidney residue concentrations reported here for May 1992 (Figure 2 and Table 6). Results from April, 1989 (Phillips and Spoon 1990), August, 1991 and November, 1991 (Phillips 1993) for brown trout from below Warm Springs include mean concentrations (μg/g dry weight) of 1635 to 2049 for liver, 2.9 to 16.6 for gill, and 8.5 to 11.4 for kidney.

Copper residue concentrations reported for fish from various sites in the United States are substantially lower than the concentrations observed in Clark Fork fish. As summarized in Table 6, Schmitt and Brumbaugh (1990) reported a national geometric mean of 3.25 μg/g and a national 85th percentile concentration of 5.0 μg/g for Cu concentration in whole fish from 109 U.S. sites (concentrations were adjusted from Schmitt and Brumbaugh's wet weight residue values to dry weight residue values). Both Turah Bridge and Warm Springs fish samples were above the national geometric mean and Warm Springs fish samples were above the 85th percentile concentrations (Table 6). Liver residue concentrations in fish from the Great Lakes ranged from 28 to 80 μg/g dry weight compared to about 1000 to 2500 μg/g dry weight reported from the upper Clark Fork River (Table 6 and Phillips 1993).

Overall, Cu residues are highly elevated compared to fish whole body residues from throughout the United States, and Cu residues in fish liver samples from the Clark Fork are 10 to 100 times higher than fish liver samples collected from the relatively uncontaminated upper Great Lakes. Moreover, these highly elevated residues in Clark Fork fish have been present for at least the past 20 years and undoubtedly since fish began to re-colonize the upper Clark Fork River during the 1960's.

Physiological Malfunctions and Structural Pathologies

In conjunction with the significantly elevated metal residue concentrations described above for adult brown trout collected from the Clark Fork River and for brown and rainbow trout early

lifestages exposed in the laboratory to water-borne and dietary metals from the Clark Fork, we evaluated physiological and structural (histopathological) changes in tissues from these same fish samples. The most marked abnormalities were observed in the concentrations of lipid peroxidation products and metallothionein and in tissue histopathology.

The concentrations of lipid peroxidation products were significantly greater in large intestine, liver and pyloric caeca tissues of adult brown trout collected from below Warm Springs than in the same tissues of fish from reference sites (Table 2). Woodward et al. (1993a) also observed significantly increased lipid peroxidation levels in early lifestage brown trout fed an invertebrate diet collected from below Warm Springs ponds. Lipid peroxidation affects the integrity of cell membranes. Cell membranes are dynamic structures that maintain fluidity so that necessary substances can freely pass through the membrane. At the same time, the structural integrity is maintained to keep other substances either outside or inside the cell and to keep membrane-bound enzymes in the proper configuration. Lipid peroxidation can compromise this balance of fluidity and structure in the cell membrane by damaging polyunsaturated fatty acids located in the cell membrane. This damage can decrease the fluidity of cell membranes, increase the leakiness of cell membranes, and inactivate membrane-bound enzymes. These changes in the structural integrity of cell membranes may ultimately result in tissue damage and cell death (Halliwell and Gutteridge 1985).

Metals such as Cu that exist in more than one valence state can be catalysts for lipid peroxidation (Wills 1985). Stern (1985) reported that copper, sulfhydryls, and oxygen appear to interact in biological systems to initiate lipid peroxidation. Radi and Matkovics (1988) measured increased lipid peroxidation in liver, gill and muscle of carp exposed to $CuSO_4$ in the water. Metals can also cause lipid peroxidation because they inhibit important antioxidant enzymes such

as glutathione peroxidase and transferase (Reddy et al. 1981, Splittgerber and Tappel 1979, DiGiulio et al. 1989).

Metallothioneins are metal binding proteins that interact primarily with Cu, Cd and Zn as well as other metals (Stegeman et al. 1992). Metallothionein concentrations in livers of brown trout collected from below Warm Springs were twice as high as those in brown trout from the combined reference sites. Woodward et al. (1993a) did not observe a significant increase in metallothionein concentrations as a result of metal exposure in their experiments, however, the small size of these fish necessitated analysis of whole fish. Because metallothionein is predominantly present in the liver and kidney tissues (Olsson and Högstrand 1987), any differences may have been masked in the Woodward et al. whole body determinations. Increased metallothionein concentrations in fish liver and gill following controlled exposures to metals have been well documented (Reichert et al. 1979, Dixon and Sprague 1981a, Spry and Wood 1989) and these increases have been associated with decreased growth (Dixon and Sprague 1981a and b, Roch and McCarter 1984). Deniseger et al. (1990) noted a parallel decline of metallothionein concentrations in the livers of fish in Buttle Lake and metals concentrations in the water. The authors suggested that the biological recovery lagged behind the improvements in water chemistry and suggested that metallothionein be monitored as an indicator of fish health. Roch et al. (1982) also documented increased metallothionein levels in fish from the Campbell River watershed, which was contaminated with metals.

Tohtz (1992) observed a high incidence of scale loss and regeneration in free-ranging brown trout in the Clark Fork River and relatively much lower incidence of this phenomenon in brown trout from Rock Creek and the Big Hole River. Farag and Bergman (1993) reported scale loss in 83% of adult rainbow trout fed a diet collected from below Warm Springs. Lead and Zn

accumulate in the scales of fish through both water exposures and interperitoneal injections (Varanasi and Markey 1978, Sauer and Watabe 1984). Sauer and Watabe (1989) used X-ray microanalysis to observe Zn incorporated into the calcified region of the circuli rather than simply being adsorbed onto the surface of scales. The authors discussed the possibility that scales act as a protective sink for metals during exposure (Sauer and Watabe 1984, Sauer and Watabe 1989). Because minerals can be resorbed from the scales into the fish blood circulation during certain time periods, such as spawning, the stored metals may be resorbed at that time (Varanasi and Markey 1978). Also, it is generally accepted in fish culture that scale loss (e.g., from excessive handling) can lead to increased external disease infection and parasite infestation, which can in turn increase morbidity and mortality (Gaines and Rogers 1975).

In addition to this major gross pathology (scale loss) observed in Clark Fork brown trout from the field (Tohtz 1992) and in rainbow trout fed Clark Fork invertebrates in the laboratory (Farag and Bergman 1993), a series of pathologies were also observed during the histopathology examination of fish tissues from the field and laboratory studies reported here. The main histopathology observations from tissues of adult brown trout from the Clark Fork River sites were Cu inclusions in hepatocyte cytoplasm and vacuolation of hepatocyte nuclei. The same but less severe histopathologies were observed in several fish from Rock Creek, and none of the fish from the Big Hole River exhibited these pathologies. The localization of Cu in hepatocyte cytoplasm from these Clark Fork brown trout emphasizes the importance of the extremely high liver Cu residues reported above for these same fish, since actual deposition of Cu intracellularly in the liver explains diversion of cell metabolism to metallothionein synthesis (significantly elevated liver metallothionein levels described above), and implies severe disruption of cell function. In fact, nuclear vacuolation observed in hepatocytes from many of these same fish is a

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sign of irreversible cell destruction and eventual cell death most likely by karyolysis (Beth MacConnell, U.S. FWS Fish Technology Center, Bozeman, MT, Personal Communication). Calventi and Nigrelli (1961) also observed vacuolation of hepatocytes in fish following exposure to waterborne Cu.

Woodward et al. (1993a) observed swelling of the pancreatic tissue, a depletion in the number of zymogen granules in the pancreatic cells and severe vacuolation and sloughing of intestinal mucosal epithelial cells of brown trout reared on an invertebrate diet collected from below Warm Springs. The impacted guts in brown trout observed by Woodward et al. (1993a) are likely due to accumulated metals in the tissues interfering with normal movement and absorption of food through the gut. For example, lead affects nervous tissue and the gastrointestinal tract of mammals in this manner (Schwartz et al. 1988, Dreisbach 1983, Scharding and Oehme 1973). All of the pathologies observed in Woodward's laboratory feeding studies would compromise the digestive function of the affected fish and help to explain the significant effects on growth (Woodward et al. 1993a) and on growth and survival (Woodward et al. 1993b) observed in the laboratory feeding studies.

Injury to Growth, Survival and Reproduction

The results from this study, on metal residue accumulation and physiological and structural abnormalities in brown trout collected from the Clark Fork River and in brown and rainbow trout exposed to water and dietary metals typical of the Clark Fork river, directly demonstrate significant effects of hazardous substances from the Clark Fork River on growth and indirectly demonstrate probable effects on survival and reproduction.

To assess the effects of Clark Fork metals on growth in fish, two sets of data are available from the field and two sets of data are available from controlled laboratory experiments

that simulated conditions in the Clark Fork River. In the field, adult brown trout were collected from each of two sites on the Clark Fork River and two reference sites. Mean length, weight and condition factors (KTL) for these fish are presented in Table 1. And although there were no significant differences (at $\alpha = 0.05$) in any of these three parameters, the KTL values (mean $\pm$ SEM) ranged in ascending order from Warm Springs (0.31 $\pm$ 0.04) < Turah Bridge (0.46 $\pm$ 0.16) < Big Hole/Rock Creek combined reference (0.69 $\pm$ 0.31). Since we did not age these fish samples to determine lengths, weights and KTL values for separate age classes, variances were large especially for the Turah Bridge and reference site samples, and we could not detect significant differences using a conservative α value of 0.05.

Tohtz (1992) collected brown trout form the Clark Fork and Big Hole Rivers and calculated lengths at annulus formation for six age classes form the Clark Fork and four age classes from the Big Hole river. In this analysis, mean length at annulus formation for age 4, 5 and 6 fish were each larger in Big Hole fish than the respective age 4, 5 and 6 fish from the Clark Fork. In two age classes (1982 and 1983) these differences were significant ($\alpha \leq 0.05$).

In the controlled laboratory experiments, Woodward et al. (1993a) observed significant reductions in growth of early lifestage brown and rainbow trout when the fish were exposed to dietary or waterborne metals from the Clark Fork River. By the termination of this 88-day experiment, the brown trout and rainbow trout fed a diet collected from near Turah Bridge outweighed the fish that were fed diets from Gold Creek or below Warm Springs by 30 - 50 percent. The magnitude of this difference was so great it could be observed by a simple visual inspection of the fish. In a second study, Woodward et al. (1993b) observed significant detrimental effects on growth and survival in rainbow trout fed an invertebrate diet collected from below Warm Springs.

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The results discussed above, on KTL measurements and calculated age class growth increments, suggest a possible growth reduction in brown trout collected from the Clark Fork River as compared to reference sites. The results presented by Woodward et al. (1993a and b), from controlled laboratory feeding studies that simulated conditions in the Clark Fork River, confirm a significant effect on growth in brown and rainbow trout when the fish were fed an invertebrate diet collected from below Warm Springs or from near Gold Creek, when compared to fish fed an invertebrate diet collected near Turah Bridge. Moreover, waterborne exposures to metals at concentrations typical of the Clark Fork exacerbated the growth reductions observed with the dietary exposures.

Taken together these observations from the field and the laboratory provide strong confirmation that metal exposure conditions in the Clark Fork River are sufficiently severe to cause injury to resident Clark Fork trout populations through reduced growth. Furthermore, if we consider the other information collected in this study along with available relevant information from the published literature, a cause-effect linkage between metal exposures typical of the Clark Fork and injury due to reduced growth is further substantiated and confirmed. For instance, for fish exposed to Clark Fork metals in the laboratory or field, we have presented results showing elevated concentrations of lipid peroxidation products, elevated concentrations of tissue metallothionein, increased scale loss, tissue histopathology in the gut and liver, and significantly elevated tissue metal residues. Of these measurements the most complete linkages available in the published literature between metal exposure and effects on growth relate to metallothionein concentrations and tissue metal residues.

Several published laboratory studies (Dixon and Sprague 1981a and b) have linked Cu exposure, elevated metallothionein, acclimation to Cu, and reduced growth in rainbow trout. In

these studies waterborne Cu concentrations that were sufficiently high to induce increased metallothionein also increased tolerance to subsequent challenges to lethal Cu exposures, demonstrating the relationship between elevated metallothionein levels and acclimation of the fish. However, in all Cu exposures where metallothionein was significantly elevated, the investigators also observed a significant reduction in growth. Dixon and Sprague (1981b) conclude that the reduced growth that they observed represented the increased metabolic costs associated with acclimation to Cu. In the Clark Fork brown trout collected from below Warm Springs, significantly elevated liver metallothionein, significantly elevated lipid peroxidation in several tissues, marked nuclear vacuolation in hepatocytes, intracellular Cu granule deposition in hepatocytes, and significantly elevated liver and whole body Cu residue accumulation, taken together, suggest a syndrome that can be associated with reduced growth.

In a series of long-term studies where fish were exposed to waterborne Cu concentrations, elevated liver Cu residues were always associated with significant effects on growth, survival or reproduction (see Table 6). In a 22-month water exposure with bluegills, Benoit (1975) documented effects on growth and reproduction with mean liver concentrations of 480 μg Cu/g (dry weight) and no effects on growth and reproduction with mean liver concentrations of 57 μg Cu/g. McKim and Benoit (1974) did not observe effects on growth and reproduction in a 24-month water exposure of brook trout with 238 μg Cu/g in livers (Table 6), but in an earlier 22-month study (McKim and Benoit 1971), using slightly higher water exposures to Cu, they observed significant effects on survival, growth and reproduction of brook trout (tissue Cu residues were not measured). Based on these studies, liver Cu concentrations between 238 and 480 μg Cu/g appear to be associated with detrimental effects on growth and reproduction. Although variables associated with water and food route exposure to Cu make it difficult to

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establish precise thresholds for effects, the liver Cu concentrations measured in brown trout collected from below Warm Springs far exceed those where effects on growth and reproduction have been demonstrated in the laboratory. Moreover, the concentrations of Cu in livers of fish from the reference sites are also above this laboratory derived "threshold" and may also be affected. This adds an additional element of conservativism to our comparison of fish populations in Clark Fork and reference sites (DCC 1993).

Other metals were also elevated in brown trout collected from the Clark Fork River from below Warm Springs. We observed significantly higher tissue concentrations of As, Cd and to lesser extent Pb in brown trout collected from the Clark Fork River as compared to fish collected from the reference sites. These additional metals may further compromise the health of fish in the Clark Fork River as researchers have linked reduced growth to increased tissue metal concentrations in the laboratory. Cockell and Hilton (1988) observed decreased survival and growth and increased concentrations of As in tissues of rainbow trout exposed to As via the diet. Benoit et al. (1976) observed elevated concentrations of Cd in gill, liver and kidney tissues along with reduced growth in brook trout exposed to Cd via the water. Holcombe et al. (1976) observed increased Pb concentrations in gill, liver and kidney tissues of first and second generation brook trout that exhibited erratic swimming behavior and scoliosis, and third generation fish from the same experiment had decreased growth.

The high concentrations of Cu and other metals in tissues of brown trout collected from the Clark Fork River indicate that the health of these fish is impaired. Metal residues measured in tissues collected from brown trout in this study have been associated with effects on growth and reproduction in published laboratory studies. In particular, the liver concentrations of Cu in fish collected from the Clark Fork River exceed a threshold where effects on growth and

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reproduction have been observed by other researchers. High metal residues associated with effects on growth were also observed in brown and rainbow trout fed an invertebrate diet collected from the Clark Fork River (Table 6 -- Woodward et al. 1993a). In addition, to the significantly elevated tissue metal residues, we have also measured other physiological malfunctions in the field and the laboratory, including elevated lipid peroxidation and metallothionein concentrations, scale loss and histological changes, which support the conclusion that fish health in the Clark Fork River is impaired resulting in effects on growth.

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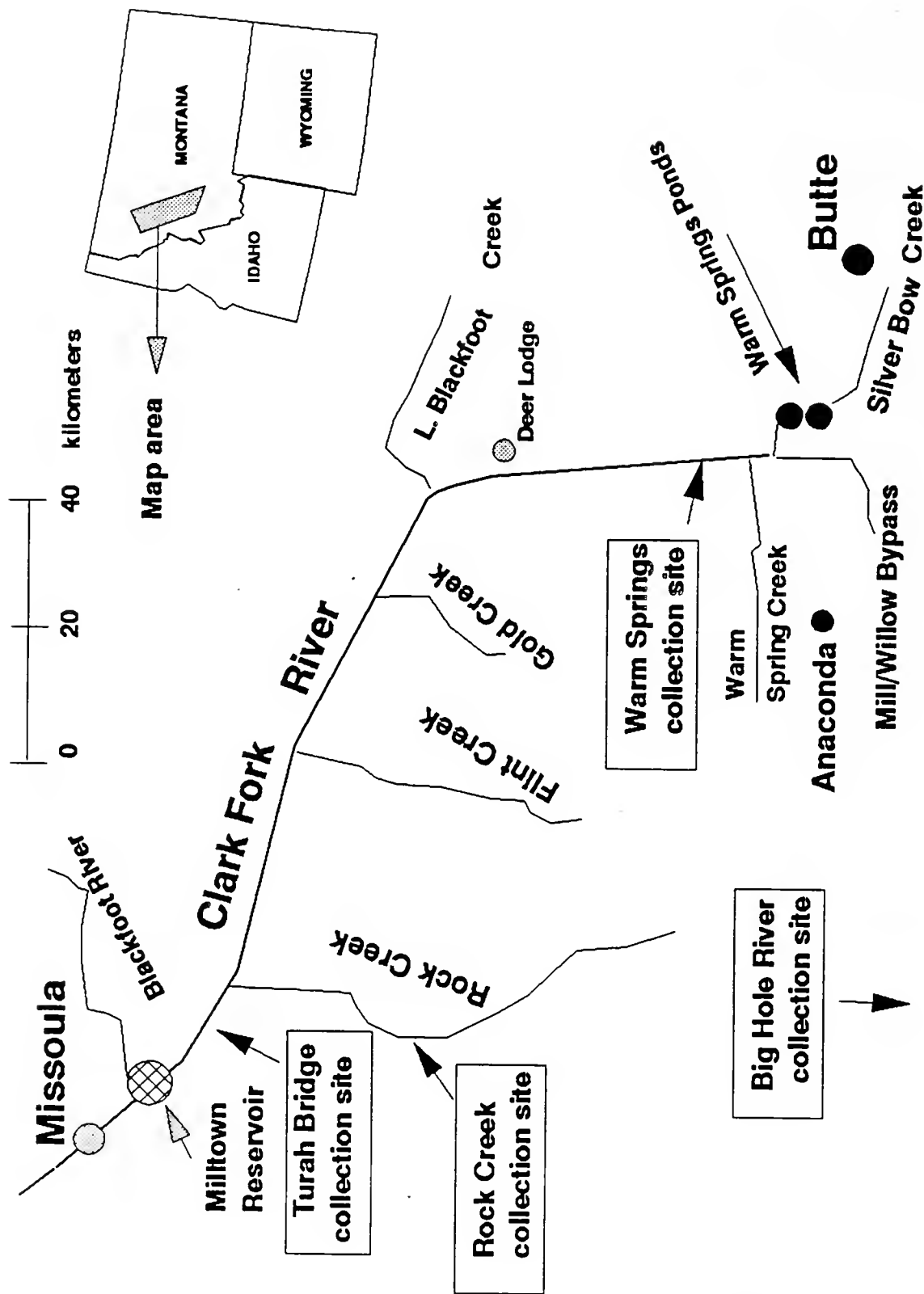
Figure 1. Upper Clark Fork River from confluence of Silver Bow Creek, Warm Springs Creek to Missoula, Montana. Adult brown trout were collected at Big Hole, Rock Creek, near Turah Bridge and below Warm Springs Ponds.

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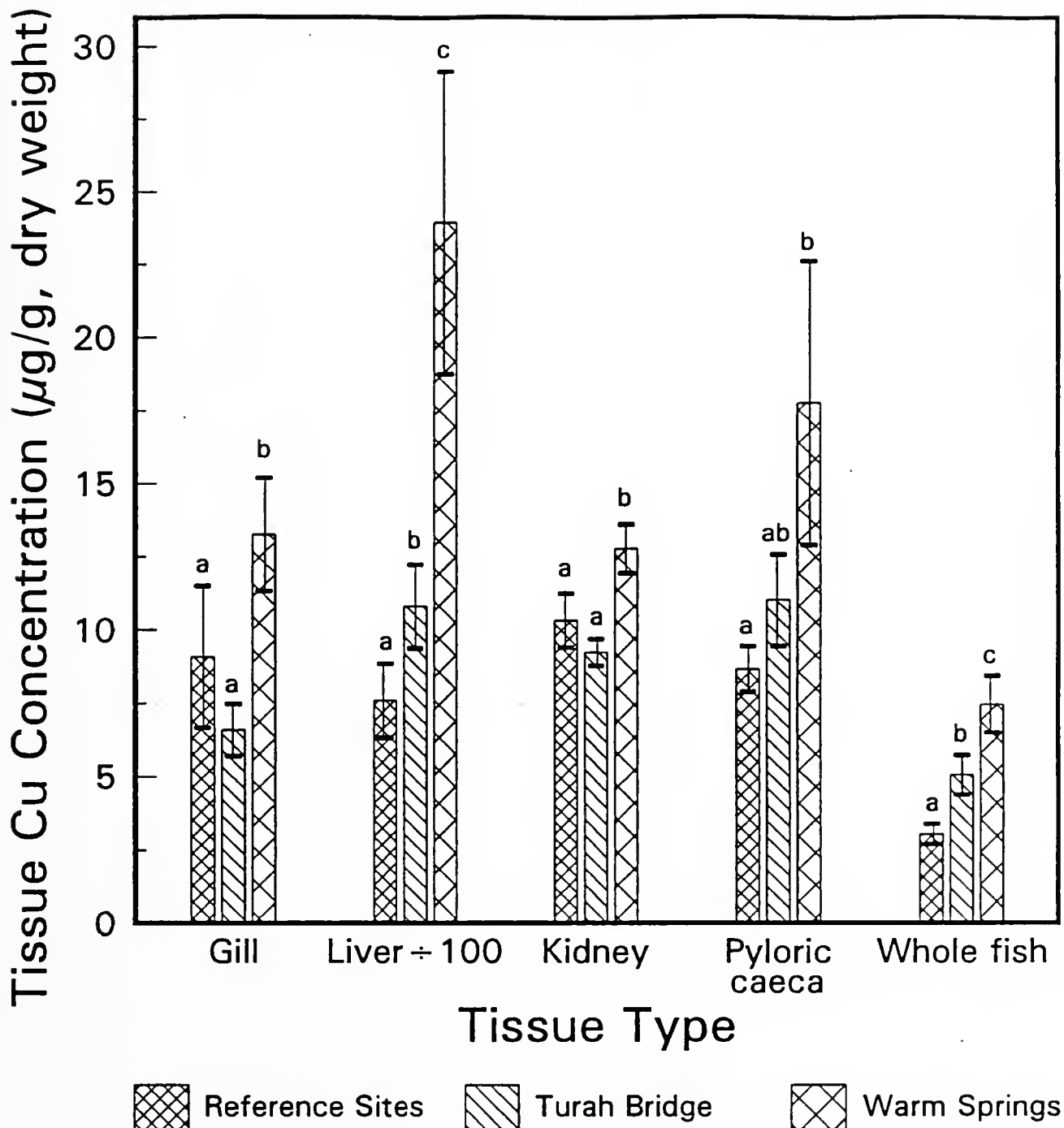
Figure 2. Copper concentrations (mean ± 1 SEM, μg/g dry wt) in tissues and whole fish for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Reference Sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at alpha = 0.05, based on the Wilcoxon rank sum test which compares median values. See Appendix Tables 1-5 for actual mean and SEM.
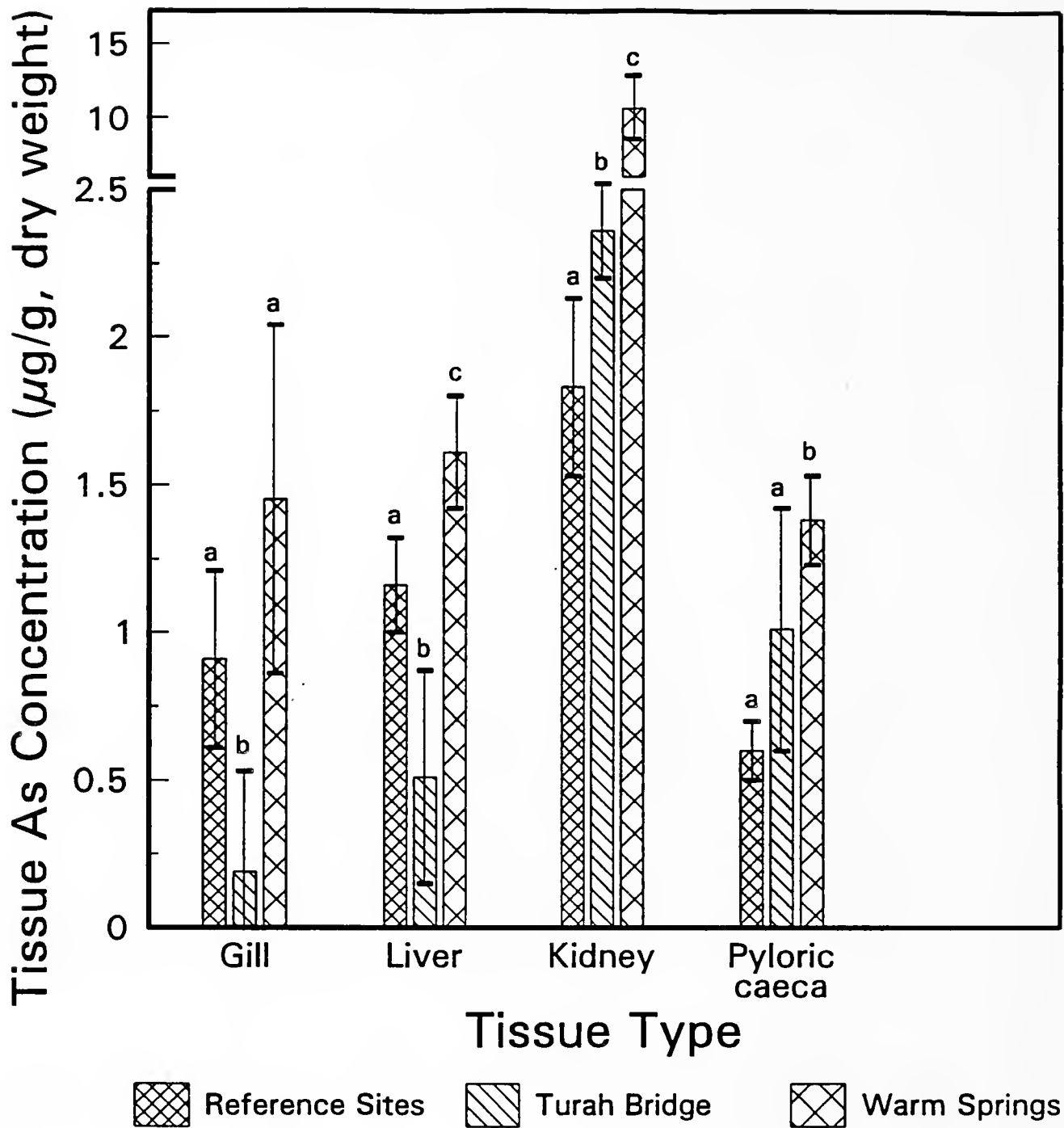
Figure 3. Arsenic concentrations (mean ± 1 SEM, μg/g dry wt) in tissues and whole fish for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Reference Sites) and residue concentration values with the same letter (within a tissue type) are not significantly different at alpha = 0.05, based on the Wilcoxon rank sum test which compares median values. See Appendix Tables 1-5 for actual mean and SEM values.
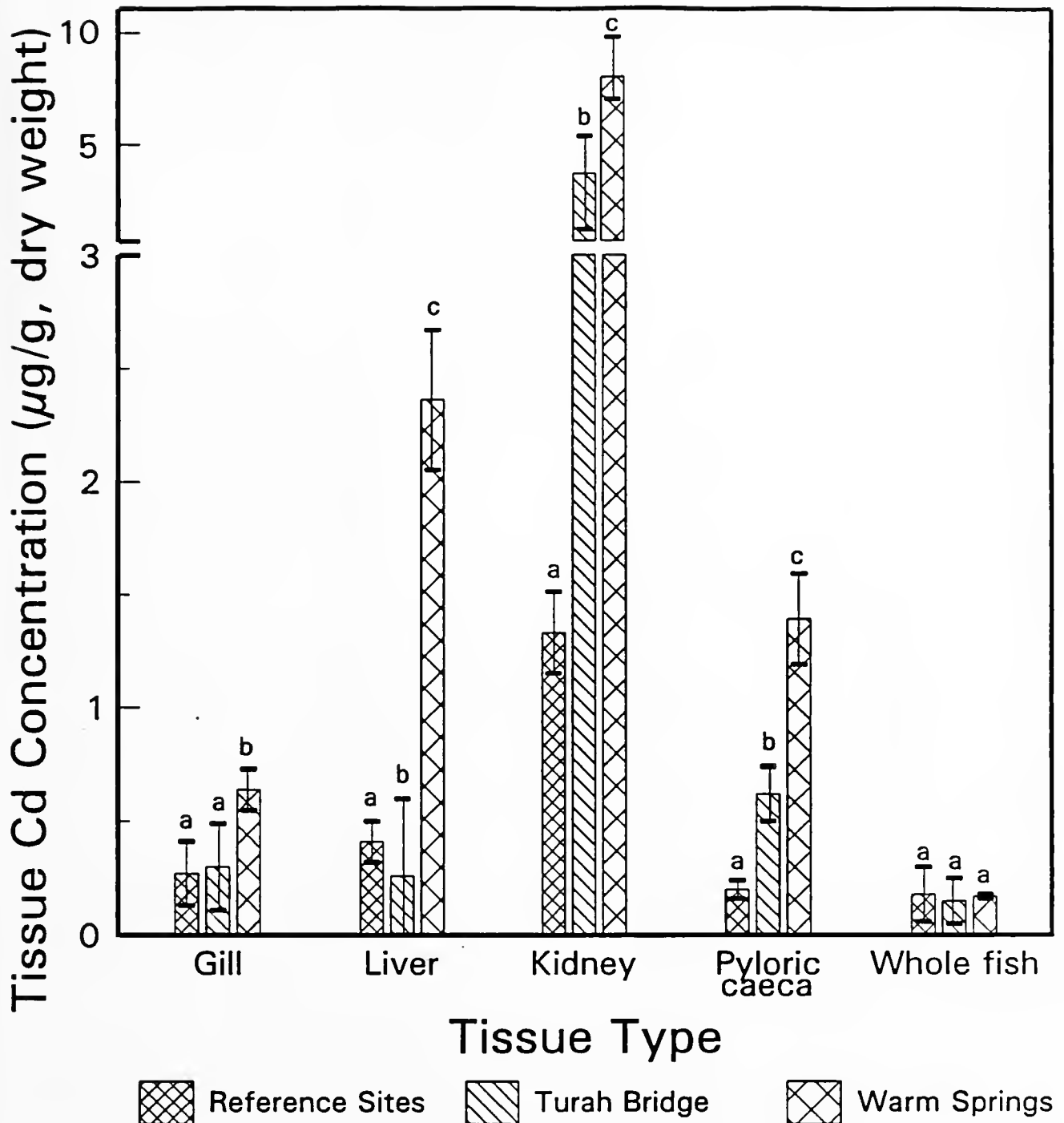
Figure 4. Cadmium concentrations (mean ± 1 SEM, µg/g dry wt) in tissues and whole fish for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Reference Sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at alpha = 0.05, based on the Wilcoxon rank sum test which compares median values. See Appendix Tables 1-5 for actual mean and SEM values.
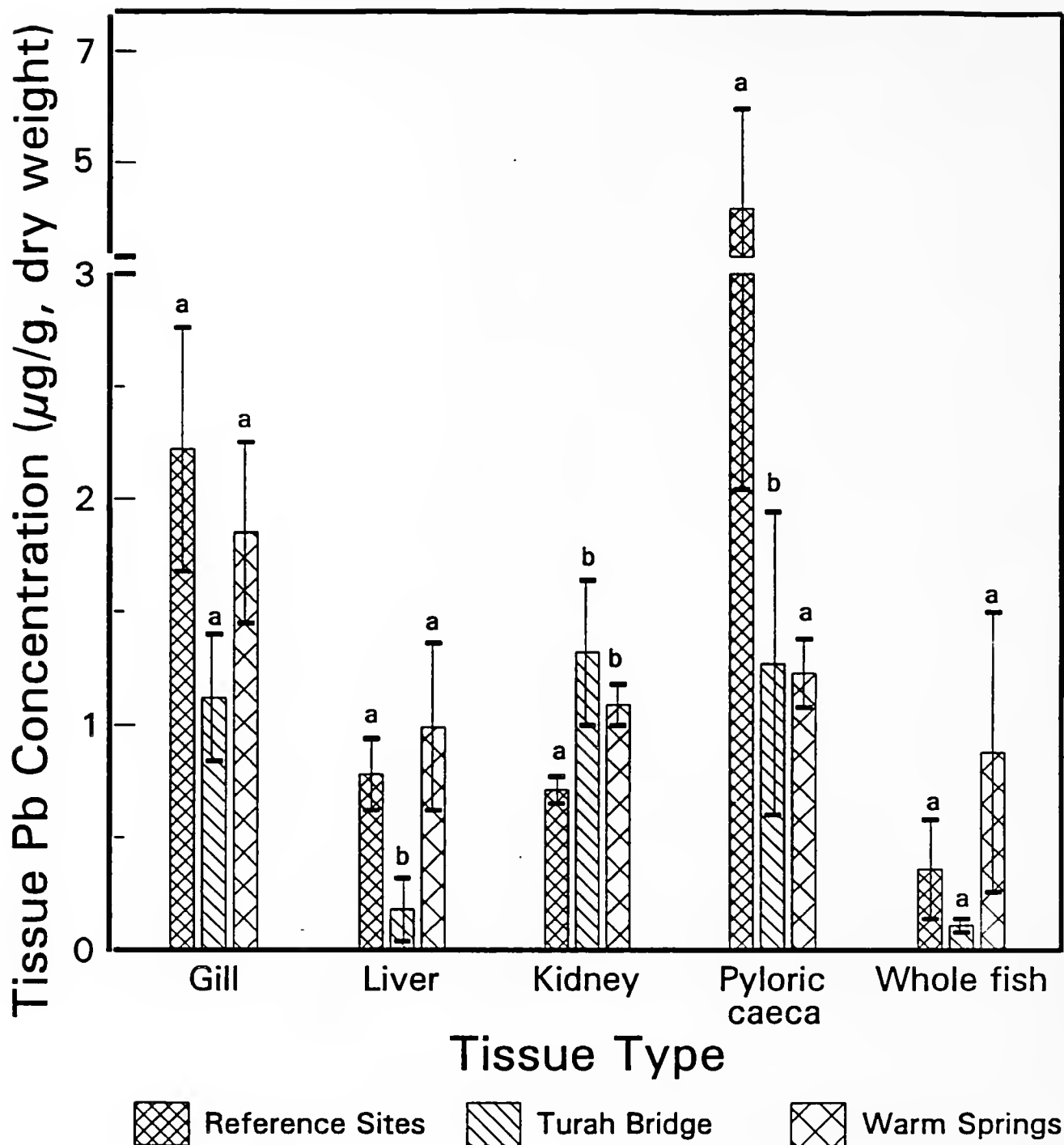
Figure 5. Lead concentrations (mean ± 1 SEM, μg/g dry wt) in tissues and whole fish for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Reference Sites) for the tissue comparisons (the reference for whole fish is the Big Hole River only), and residue concentration values with the same letter (within a tissue type) are not significantly different at alpha = 0.05, based on the Wilcoxon rank sum test which compares median values. See Appendix Tables 1-5 for actual mean and SEM values.

Table 1. Mean ($\pm$ 1 SEM) weight (g), length (mm) and condition factor (KTL = Weight x 10^5/Length^3) of adult brown trout collected from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference) for the means comparisons, and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Weight (g)	Length (mm)	KTL
Reference Sites				
Rock Creek	9	647 (104)	397 (26)	0.93 (0.64)
Big Hole	10	562 (56)	377 (16)	0.41 (0.14)
Combined Reference	19	603[a] (57)	386[a] (15)	0.69[a] (0.31)
Clark Fork Sites				
Turah Bridge	9	644[a] (93)	400[a] (24)	0.46[a] (0.16)
Warm Springs	10	596[a] (40)	396[a] (8.9)	0.31[a] (0.04)

Table 2. Lipid peroxidation of tissues (means ± SEM) sampled from adult brown trout from the Clark Fork and reference sites during May, 1992. Lipid peroxidation is expressed as the flourometric measurement (relative intensity) of a chloroform extract of tissue. The relative intensity was measured at 360 nm excitation and 425 nm emission when 1.0 μg/ml of quinine sulfate measured 12.5. Rock Creek and Big Hole values were combined (Combined Reference) for the means comparisons, and means with the same letter (within a tissue) are not significantly different at alpha = 0.05.

Relative Intensity

Site	N	Gill	N	Kidney	N	Large Intestine	N	Liver	N	Pyloric Caeca	N	Stomach
Reference Sites												
Rock Creek	8	0.16 (0.01)	7	0.66 (0.05)	8	0.12 (0.03)	8	0.05 (0.02)	8	0.02 (0.01)	9	0.20 (0.02)
Big Hole	9	0.18 (0.02)	8	0.92 (0.15)	10	0.25 (0.03)	10	0.06 (0.02)	8	0.07 (0.02)	10	0.23 (0.03)
Combined Reference	17	0.17ª (0.01)	15	0.80ª (0.09)	18	0.19ª (0.03)	18	0.06ª (0.01)	16	0.05ª (0.01)	19	0.22ª (0.02)
Clark Fork Sites												
Turah Bridge	8	0.23ª (0.03)	7	0.86ª (0.07)	9	0.27ª (0.06)	8	0.08ª (0.02)	9	0.07ª (0.02)	9	0.21ª (0.04)
Warm Springs	9	0.20ª (0.03)	9	0.77ª (0.04)	10	0.36ᵇ (0.02)	10	0.27ᵇ (0.03)	10	0.21ᵇ (0.02)	10	0.23ª (0.03)

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Table 3. Metallothionein concentrations (mean ± SEM, μg/g wet weight) in liver samples of adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference) for the means comparisons, and means with the same letter are not significantly different at alpha =0.05.

		Metallothionein (μg/g)
Site	N	Liver
Reference Sites		
Rock Creek	7	168.0 (18.4)
Big Hole	9	191.7 (39.0)
Combined Reference	16	181.3[a] (22.9)
Clark Fork Sites		
Turah Bridge	7	132.8[a] (15.0)
Warm Springs	9	422.0[b] (128.7)

Table 4. Serum ion concentrations (mean ± SEM, μeq/L) of adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter designation (within an ion) are not significantly different at alpha = 0.05.

Site	N	Serum ion concentration (μeq/L)		
		Ca	K	Na
Reference Sites				
Rock Creek	8	3.29 (0.13)	37.68 (1.02)	113.98 (1.50)
Big Hole	10	3.22 (0.13)	35.87 (1.48)	107.67 (1.71)
Combined Reference	18	3.25[a] (0.09)	36.67[a] (0.94)	110.48[a] (1.36)
Clark Fork Sites				
Turah Bridge	9	3.65[a] (0.22)	38.17[a] (2.15)	98.30[b] (3.37)
Warm Springs	10	3.04[b] (0.09)	38.87[a] (1.21)	108.02[a] (1.98)

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Table 5. Stress proteins (means $\pm$ SEM, % of marker) for liver and gill of adult brown trout from the Clark Fork and reference sites during May, 1992. Means with the same letter designation (within a tissue) are not significantly different at alpha = 0.05.

Stress Proteins (% of marker)

Site	N	Tissue	Brown trout
Rock Creek	5	Liver	68.56[a] (11.48)
Warm Springs	5	Liver	79.84[a] (21.46)
Rock Creek	5	Gill	76.56[a] (19.30)
Warm Springs	5	Gill	36.64[a] (11.91)

Table 6. Comparison of tissue residue concentrations of copper in fish from the Clark Fork River and reference sites with representative background samples and laboratory effect studies.

Sample description	Tissue residues (μg/g dry wt)				Comments	Reference
	Whole Fish	Liver	Gill	Kidney		
This study[a]						
Reference	3.04	758.86	9.08	10.30	Brown trout adults	This study[a]
Turah Bridge	4.26[b]	1078.80[b]	6.59	9.22	Brown trout adults	This study[a]
Warm Springs	6.36[b]	2393.57[b]	13.26[b]	12.76[b]	Brown trout adults	This study[a]
Background						
National geometric mean	3.25[c]	---	---	---	315 samples, 109 sites, mixed species	Schmitt & Brumbaugh 1990
National 85th percentile	5.00[c]	---	---	---	315 samples, 109 sites, mixed species	Schmitt & Brumbaugh 1990
Lake Superior	---	28	---	---	Lake trout	Lucas et al. 1970
Lake Michigan	---	21	---	---	Lake trout	Lucas et al. 1970
Lake Huron	---	80[c]	---	---	Several species	Brown & Chow 1977

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Table 6 (cont.). Comparison of tissue residue concentrations of copper in fish from the Clark Fork River and reference sites with representative background samples and laboratory effect studies.

Sample description	Tissue residues (μg/g dry wt)				Comments	Reference
	Whole Fish	Liver	Gill	Kidney		
Laboratory Effect Studies						
88-d water/food exp. brown trout alevins	6.4-44.7	147-268	---	---	Range of concentrations with significant effects on growth	Woodward et al. 1993a
88-d water/food exp. rainbow trout alevins	15.9-22.5	88-125	---	---	Range of concentrations with significant effects on growth	Woodward et al. 1993a
22-mo water exp. bluegill adults (162 μg/l)	---	480	13	44	Significant effects on survival, growth and reproduction	Benoit 1975
22-mo water exp. bluegill adults (77 μg/l)	---	57	6	12	No significant effects on survival, growth or reproduction	Benoit 1975
24-mo water exp. brook trout adults (9.4 μg/l)	---	238	6.7	16.5	No significant effect on survival, growth or reproduction	McKim & Benoit 1974

[a]See Appendix Tables 1,2,3,4,5
[b]Significantly greater than reference site ($p \leq 0.05$).
[c]Copper concentrations reported on a wet weight basis multiplied by five to estimate dry weight concentrations (assumes fish are 80% water).

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Appendix Table 1. Metal concentrations (mean $\pm$ 1 SEM, $\mu g/g$ dry wt.) in gill for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

| Site | N | Gill metal concentration ($\mu g/g$ dry weight) | | | |
		Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	10.95 (5.11)	0.95 (0.55)	0.37 (0.22)	2.19 (1.03)
Big Hole	10	7.39 (0.71)	0.87 (0.29)	0.17 (0.16)	2.25 (0.48)
Combined Reference	19	9.08[a] (2.41)	0.91[a] (0.30)	0.27[a] (0.14)	2.22[a] (0.54)
Clark Fork Sites					
Turah Bridge	9	6.59[a] (0.89)	0.19[b] (0.34)	0.30[a] (0.19)	1.12[a] (0.28)
Warm Springs	10	13.26[b] (1.94)	1.45[a] (0.59)	0.64[b] (0.09)	1.85[a] (0.40)

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Appendix Table 2.   Metal concentrations (mean $\pm$ 1 SEM, μg/g dry wt.) in liver for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Liver metal concentration (μg/g dry weight)

Site	N	Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	769.60 (255.86)	0.73 (0.17)	0.18 (0.06)	0.84 (0.27)
Big Hole	10	749.19 (89.43)	1.54 (0.20)	0.62 (0.12)	0.73 (0.19)
Combined Reference	19	758.86[a] (126.01)	1.16[a] (0.16)	0.41[a] (0.09)	0.78[a] (0.16)
Clark Fork Sites					
Turah Bridge	9	1078.80[b] (142.77)	0.51[b] (0.36)	0.26[b] (0.34)	0.18[b] (0.14)
Warm Springs	10	2393.57[c] (520.03)	1.61[c] (0.19)	2.36[c] (0.31)	0.99[a] (0.37)

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Appendix Table 3. Metal concentrations (mean ± 1 SEM, μg/g dry wt.) in kidney for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Kidney metal concentration (μg/g dry weight)			
		Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	10.64 (1.57)	1.08 (0.22)	0.80 (0.14)	0.76 (0.10)
Big Hole	10	10.00 (1.11)	2.49 (0.44)	1.82 (0.23)	0.66 (0.07)
Combined Reference	19	10.30[a] (0.92)	1.83[a] (0.30)	1.33[a] (0.18)	0.71[a] 0.06)
Clark Fork Sites					
Turah Bridge	9	9.22[a] (0.45)	2.36[b] (0.16)	4.39[b] (0.86)	1.32[b] (0.32)
Warm Springs	10	12.76[b] (0.84)	11.29[c] (2.07)	8.56[c] (0.90)	1.09[b] (0.09)

Appendix Table 4.   Metal concentrations (mean ± 1 SEM, μg/g dry wt.) in pyloric caeca for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Pyloric caeca metal concentration (μg/g dry weight)			
		Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	9.01 (1.48)	0.33 (0.08)	0.13 (0.02)	7.19 (4.08)
Big Hole	10	8.34 (0.70)	0.85 (0.14)	0.26 (0.07)	1.21 (0.14)
Combined Reference	19	8.66[a] (0.77)	0.60[b] (0.10)	0.20[a] (0.04)	4.04[a] (2.00)
Clark Fork Sites					
Turah Bridge	9	11.00[ab] (1.57)	1.01[a] (0.41)	0.62[b] (0.12)	1.27[b] (0.67)
Warm Springs	10	17.74[b] (4.85)	1.38[b] (0.15)	1.39[c] (0.20)	1.23[a] (0.15)

Appendix Table 5. Metal concentrations (mean $\pm$ 1 SEM, μg/g dry wt.) in whole fish for adult brown trout from the Clark Fork and reference sites during May, 1992. Means with the same letter are not significantly different at alpha = 0.05.

Site	N	Whole body metal concentration (μg/g dry weight)		
		Cu	Cd	Pb
Reference Site				
Big Hole	10	3.04[a] (0.35)	0.18[a] (0.12)	0.36[a] (0.22)
Clark Fork Sites				
Turah Bridge	9	4.26[b] (0.96)	0.14[a] (0.08)	0.13[a] (0.03)
Warm Springs	10	6.36[c] (1.34)	0.15[a] (0.02)	0.74[a] (0.53)

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Appendix Table 6. Metal concentrations (mean ± 1 SEM, μg/g wet wt.) in gill for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Gill metal concentration (μg/g wet weight)			
		Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	1.83 (0.88)	0.16 (0.09)	0.06 (0.04)	0.37 (0.18)
Big Hole	10	1.24 (0.12)	0.15 (0.05)	0.03 (0.03)	0.38 (0.08)
Combined Reference	19	1.52[a] (0.41)	0.15[a] (0.05)	0.04[a] (0.02)	0.37[a] (0.09)
Clark Fork Sites					
Turah Bridge	9	0.97[a] (0.11)	0.03[b] (0.06)	0.05[a] (0.03)	0.16[b] (0.04)
Warm Springs	10	2.30[b] (0.35)	0.25[a] (0.10)	0.11[b] (0.02)	0.32[a] (0.07)

Appendix Table 7.   Metal concentrations (mean $\pm$ 1 SEM, µg/g wet wt.) in liver for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Liver metal concentration (µg/g wet weight)			
		Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	180.07 (57.41)	0.17 (0.04)	0.04 (0.01)	0.20 (0.07)
Big Hole	10	170.20 (21.22)	0.35 (0.04)	0.14 (0.03)	0.16 (0.04)
Combined Reference	19	174.88[a] (28.53)	0.27[a] (0.04)	0.09[a] (0.02)	0.18[a] (0.04)
Clark Fork Sites					
Turah Bridge	9	261.53[b] (32.49)	0.12[b] (0.08)	0.06[b] (0.08)	0.04[b] (0.03)
Warm Springs	10	575.34[c] (115.52)	0.39[c] (0.04)	0.57[c] (0.07)	0.23[a] (0.08)

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Appendix Table 8. Metal concentrations (mean $\pm$ 1 SEM, μg/g wet wt.) in kidney for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	2.08 (0.35)	0.21 (0.05)	0.15 (0.03)	0.15 (0.02)
Big Hole	10	2.01 (0.23)	0.50 (0.09)	0.37 (0.05)	0.13 (0.01)
Combined Reference	19	2.04ᵃ (0.20)	0.36ᵃ (0.06)	0.27ᵃ (0.04)	0.14ᵃ (0.01)
Clark Fork Sites					
Turah Bridge	9	1.81ᵃ (0.09)	0.46ᵇ (0.04)	0.85ᵇ (0.16)	0.26ᵇ (0.06)
Warm Springs	10	2.66ᵇ (0.18)	2.36ᶜ (0.45)	1.78ᶜ (0.19)	0.23ᵇ (0.02)

Kidney metal concentration (μg/g wet weight)

Appendix Table 9. Metal concentrations (mean ± 1 SEM, μg/g wet wt.) in pyloric caeca for adult brown trout from the Clark Fork and reference sites during May, 1992. Rock Creek and Big Hole values were combined (Combined Reference), and means with the same letter are not significantly different at alpha = 0.05.

Site	N	Cu	As	Cd	Pb
Reference Sites					
Rock Creek	9	2.36 (0.25)	0.09 (0.25)	0.04 (0.00)	1.81 (1.00)
Big Hole	10	2.44 (0.17)	0.25 (0.04)	0.08 (0.02)	0.35 (0.04)
Combined Reference	19	2.40[a] (0.14)	0.17[a] (0.03)	0.06[a] (0.01)	1.04[a] (0.49)
Clark Fork Sites					
Turah Bridge	9	3.32[ab] (0.52)	0.29[a] (0.11)	0.19[b] (0.04)	0.33[b] (0.15)
Warm Springs	10	5.15[b] (0.99)	0.44[b] (0.05)	0.41[c] (0.05)	0.41[ab] (0.07)

Pyloric caeca metal concentration (μg/g wet weight)

Appendix Table 10. Autopsy assessment parameters measured on adult brown trout collected in the field May, 1992. RC = Rock Creek site, BH = Big Hole site, TB = Turah Bridge site, and WS = Warm Springs site. KTL = the condition factor calculated as (Weight x 10^5)/Length^3. EYE, GILL, SPL = Spleen, KID = Kidney, LIV = Liver, FIN, OPL = Opercular, and OTHER are all expressed as percent of the fish scoring a normal value. BIL = Bile and FAT expressed as a mean index of observations. For bile, the larger index denotes the more green present in the bile, for fat, the larger index denotes a higher percent fat covering the pyloric caeca. N is 8 to 10 for all measurements and standard deviation is in parentheses.

| SITE | AUTOPSY PARAMETER EXPRESSED IN PERCENT NORMAL OR AVERAGE INDEX | | | | | | | | | | | | | |
	KTL	EYE	GILL	PABR	THY	FAT	SPL	H. GUT	KID	LIV	BIL	FIN	OPL	OTHER
RC	0.93	100	100	89	0.89	1.00	11	100	100	44	0.67	78	100	89
	(1.92)				(0.93)	(0.50)					(0.71)			
BH	0.48	100	100	100	0.40	1.50	44	100	100	100	0.30	100	100	100
	(0.45)				(0.70)	(0.53)					(0.48)			
TB	0.46	100	100	78	0.11	1.56	22	100	100	78	0.44	100	100	78
	(0.48)				(0.33)	(1.01)					(0.53)			
WS	0.31	90	100	89	0.50	1.60	50	100	100	40	0.20	100	100	90
	(0.12)				(0.53)	(0.70)					(0.42)			

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AQUATIC RESOURCES INJURY REPORT

APPENDIX G

Assessment of Injury to Fish Populations: Clark Fork River NPL Sites, Montana

Prepared by:

Don Chapman Consultants, Inc.

ASSESSMENT OF INJURY TO FISH POPULATIONS:

CLARK FORK RIVER NPL SITES, MONTANA

Prepared for

RCG/Hagler, Bailly, Inc.
P.O. Box Drawer O
Boulder, Colorado 80306-1906

Prepared by

Don Chapman
consultants inc.

3653 Rickenbacker, Ste 200 • Boise, Idaho 83705
(208) 383-3401 • FAX (208) 344-4861

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EXECUTIVE SUMMARY

We compared trout numbers and biomasses in the upper Clark Fork River and Silver Bow Creek (test sites) with reference sites substantially unaffected by hazardous substances. By pairing test and reference sites with stream classification, we removed most of the variability caused by geology, land type, valley bottom type, and land and water uses. Thus, we compared paired sites classed as alike in our stratification scheme. We found that reference sites contained significantly more trout per unit area (4.5-fold more adult trout)[1] than did the sites in the Clark Fork River. No trout lived in Silver Bow Creek. We concluded that the differences between test and reference sites show real and statistically-significant effects of hazardous substances in the test sites. Biomasses of trout in reference sites also significantly exceeded those in test sites. The superiority of reference sites held for both brown (Salmo trutta) and rainbow trout (Oncorhynchus mykiss).

MAIN CONCLUSION: Heavy metals in the Clark Fork River and Silver Bow Creek greatly reduce brown and rainbow trout numbers and biomass in those waters.

We found some significant differences between some components of habitat in some test and reference sites. We used physical habitat simulation (PHABSIM) modeling within the Instream Flow Incremental Methodology (IFIM) to adjust for habitat differences at summer flow in test and reference sites. After adjusting for habitat differences and flows,

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[1] In this report, differences in mean densities and biomasses represent the magnitude of the differences between mean scores in test and reference sampling sites; they have not been weighted for total areas of states or reaches.

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we still found that all trout and adult trout were significantly more abundant in reference sites than in test sites.

KEY CONCLUSION: After adjustment for habitat differences between Clark Fork test sites and reference sites in other streams, we found all trout and adult trout were about two-fold more abundant in reference sites.

Biomass of trout in reference sites significantly exceeded that in test sites. After we adjusted for habitat and flow differences between test and reference sites, we found that trout biomass in the latter significantly exceeded that in the former. Adult trout biomass in the reference sites, with habitat and flow differences adjusted, significantly exceeded that in reference sites.

KEY CONCLUSION: After we adjusted for habitat differences, we found biomasses of all trout and adult trout in reference sites exceeded those in Clark Fork test sites by about two-fold.

Juvenile trout (2-8 inches) made up 66% of all trout in test states and 63% of trout in reference sites. They were significantly more abundant in reference sites. After we adjusted for habitat and flow differences, we found densities of juveniles in reference sites significantly exceeded those in Clark Fork test sites. In both test and reference sites, juveniles made up about 15% of total trout biomass. Juvenile biomasses were, on average, 3.0 times greater in reference sites.

KEY CONCLUSION: Juvenile trout were much more abundant and had greater biomass in reference sites than in Clark Fork test sites.

No trout lived in Silver Bow Creek, while reference sites outside Silver Bow Creek supported rainbow trout (<u>Oncorhynchus mykiss</u>), brown trout (<u>Salmo trutta</u>), or brook trout (<u>Salvelinus fontinalis</u>). About 29 miles of Silver Bow Creek contain no trout.

KEY CONCLUSION: Silver Bow Creek does not support trout in its present polluted state.

Our habitat evaluations indicated that the Clark Fork River contained more suitable habitat per unit area than reference sites, on average. The Clark Fork has a relatively high specific conductance, an indicator of dissolved salts. These two factors should cause the Clark Fork to contain a higher standing crop of trout than do reference sites.

KEY CONCLUSION: The Clark Fork River should contain more trout than reference sites, on average, because it contains more habitat and higher dissolved salts.

Standing crops of adult brown trout in the Clark Fork sites, averaging 21.6% of the adult numbers in reference sites, should cause reduced angler catch rates, apart from effects on angler visits. Catch rates and total sustainable harvest should rise more or less linearly on average with standing stock of fish. Angler visits should correlate positively with stock and catch rates. Thus, low standing stocks of trout in the Clark Fork

River, absence of trout in Silver Bow Creek, reduce angler interest and use.

KEY CONCLUSION: Low trout standing crop in the Clark Fork River reduces angler catch, interest, and use of the river.

INTRODUCTION

Fish in the upper Clark Fork River have been, and continue to be, exposed to hazardous substances by direct exposure to contaminated surface water and sediments, and through food-chain exposures to contaminated prey. For nearly a century the upper river contained no trout because of the hazardous materials released by mining, milling, and smelting operations (Johnson and Schmidt 1988). Trout appeared in the river in the late 1950s, but populations of brown trout (Salmo trutta) did not establish until the 1970s. Numerous fish kills have occurred since the trout appeared. Averett (1961) reported several kills in the middle Clark Fork River between 1958 and 1960. Fish kills, periods of red water, and elevated metals concentrations were documented throughout the 1960's and early 1970's. Improvements in the waste treatment capabilities at Butte during the mid-1970's caused water quality to improve, resulting in an increase in brown trout numbers immediately downstream of the Warm Springs Ponds. Even with these improvements, however, fish kills have occurred frequently; eight were documented between 1983 and 1992, some killing several thousand fish (Johnson and Schmidt 1988). Trout have also been shown to have elevated concentrations of hazardous substances in their liver and kidneys. For example, Phillips and Spoon (1990) reported copper concentrations as high as 1,663 ppm in liver, 700 ppm of zinc in gill tissue, and 5.5 ppm of cadmium in kidney tissue. Silver Bow Creek still lacks trout because of releases of hazardous substances.

The brown trout is the most abundant salmonid in the upper Clark Fork between Butte and Rock Creek (Knudson 1984; Johnson and Schmidt 1988). In the late 1980s their numbers were close to 2,000 trout per mile immediately downstream from Warm Springs Ponds. Farther downstream, however, fish numbers dropped drastically, with less than 500 trout per mile around Deer Lodge and 50 trout per mile between Drummond

and the confluence with Rock Creek (Workman 1985; Johnson and Schmidt 1988). Rainbow trout (<u>Oncorhynchus mykiss</u>) are most abundant in the Clark Fork River downstream from Rock Creek, although a few appear downstream from Warm Springs Creek. Bull trout (<u>Salvelinus confluentus</u>) and westslope cutthroat trout (<u>S</u>. <u>clarki</u>) have virtually disappeared from the Clark Fork River (Knudson 1984; Montana Dept. Natural Resources 1988). In contrast, the Blackfoot River supports populations of brown, rainbow, cutthroat, bull, and brook trout (<u>S</u>. <u>fontinalis</u>) (Knudson 1984); Willow Creek supports brook and cutthroat trout; German Gulch Creek supports westslope cutthroat, brook, and brown trout; and Blacktail Creek supports brook trout (Camp, Dresser, & McKee 1991).

Stream sections classified as similar, based upon physical and chemical stream descriptors, have similar biotic communities. This does not mean identical species in all similar communities, but rather corresponding species in identical functional groups (Pennak 1971). Cairns et al. (1973) believe that stream community structure cannot be modified or altered without also changing function. Winget (1985) noted that changes to stream ecosystems included shifts in relative numbers of individuals among species, alterations in rates and quantities of material cycles including energy flow, and changes in species composition. Thus, in two ecologically similar stream sections, the magnitude of an impact on a stream community in one section can be assessed relative to the other.

We tested the hypothesis that fish abundance in the Clark Fork test sites did not differ from those in reference sites. To do this, we selected reference stream segments that had ecological and geological stream descriptors similar to those in corresponding stream segments in the Clark Fork River and Silver Bow Creek, but which were not subjected to release of hazardous substances. The matching of test and reference stream sections provided a means of separating impacts related to release of hazardous substances from damages caused by other land use activities (e.g., irrigation and

agriculture, grazing, and channelization). Our specific objective was to compare and contrast densities and biomasses of trout in test and reference sites.

METHODS

To assess damages to the Clark Fork fisheries, we first identified distinctive ecologic, geologic, geomorphic, hydrologic, and state or condition in segments of the Clark Fork River (from Milltown Dam upstream to Warm Springs liming ponds) and Silver Bow Creek (from Warm Springs liming ponds upstream to Butte). The U.S. Department of the Interior (DOI) Rules and Regulations [CFR 43 § 11.72 (d)] state that "where historical data are not available for the assessment area or injured resource...baseline data should be collected from control areas...the baseline shall be defined by field data from the control areas." DOI also states that "one or more control areas shall be selected based upon their similarity to the assessment area and lack of exposure to the discharge or release" [CFR 43 § 11.72 (d)(1)], and that "the comparability of each control area to the assessment areas shall be demonstrated, to the extent technically feasible..."[CFR 43 § 11.72 (d)(3)]. Therefore, we used the same techniques to identify sections of other streams (reference streams) that corresponded to discrete segments in the Clark Fork River and Silver Bow Creek (test streams), but that had no release of hazardous substances. Within each selected stratum or section, we randomly chose four 100-m sites in which we assessed densities and biomasses of trout. We selected one of the four sites in each stratum to assess microhabitat differences between test and reference sections. We used that information to standardize density and biomass data.

Stream Classification.

We used a hierarchical classification that identified reaches and subreaches of distinctive form, function, and ecological potential. The classification consisted of six levels (Table 1). Classes of the top levels consisted of large areas that we described based on regional criteria from small scale maps and general information sources. At successively lower levels, these areas were divided into smaller areas that were described based on criteria from large scale maps and on quantitative information. We applied the classification from the top level down, thus accounting for variance at the broadest level possible.

Ecoregions.--This is the broadest level of classification (Omernik 1987). It is based on factors (e.g., climate) that cause regional variation in ecosystems or on factors that integrate the causes of regional variations. Principal factors that we used to identify ecoregions were land surface form, potential natural vegetation, land use, and soils.

The Clark Fork Basin lies within the mountainous semiarid steppe division of the dry domain (Bailey 1981). We identified two ecoregions in the Clark Fork Basin: Northern Rockies Ecoregion (from Milltown Dam to just upstream from the Tigh Creek confluence) and the Montana Valley and Foothill Prairies Ecoregion (from just upstream from the Tigh Creek confluence to Butte) (Figure 1).

Geologic Districts.--These are areas of similar rock types or parent materials that are generally associated with distinctive structural features and areas of similar hydrographic character. Structural features are the templates on which streams have etched drainage patterns. The hydrologic character of landscapes is also influenced by the degree to which parent material has been weathered and the water-handling characteristics of the parent rock and its weathering products. Geologic districts do not change to other types in response to land uses, and they include both uplands and bottomlands. We used a 1:500,000 scale geologic map of Montana (Ross et al. 1955) to

Table 1.--Hierarchical levels of classification.

Hierarchical Level	Description
Ecoregion	An area determined by similar land-surface form, potential natural vegetation, land-use and soil (Omernik 1987); they may contain few to many geologic districts.
Geologic District	A portion of an ecoregion with relatively homogeneous parent materials, distinguished from surrounding districts by structure, degree of weathering, dominant size-fractions of weathering products and water-handling characteristics; includes both uplands and bottomlands; it may contain one to several landtype associations.
Landtype Association	Some part (or all) of a geologic district that is distinguished by a dominant geomorphic mechanism (e.g., glacial, fluvial, alluvial, lacustrine); includes both uplands and bottomlands; it contains several landtypes.
Landtype	A portion of a landtype association distinguished by form and position; it corresponds with associations of soils and plant communities; riverine-riparian habitat is contained within the valley-bottom landtype, which may include few to several valley-bottom types.
Valley-Bottom Type	A subset of the valley-bottom landtype distinguished by form, structure and the manner in which water and sediments move through the system; they are generally distributed in a predictable manner along the elevational gradient of watersheds; it contains several to many landforms.
State Type	A part of the valley-bottom type distinguished by the condition of the stream and its banks (e.g., eroded banks, laid-back banks, channelized, braided, etc.).

identify geologic districts.

We identified five geologic districts in the Clark Fork Basin: resistant sedimentary rock (mostly Paleozoic and Precambrium meta-sedimentary rock), soft sedimentary rock (mostly mesozoic sedimentary rock of marine origin), Tertiary alluvium (weakly consolidated valley-fill deposits), volcanic rock (Tertiary and Mesozoic age), and granite (Tertiary and Cretaceous age) (Figure 2).

Ecoregions of Western Montana

Figure 1

N
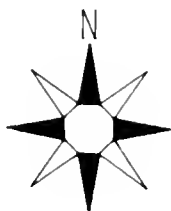
| 0 | 50 | 100 | 150 |

Miles
Scale 1:3,000,000

Black Fork

Flint

Cr

Big Hole River

Ruby River

Northern Rockies

Montana Valley and
Foothill Prairies

Northern Montana
Glaciated Plains

Northwestern
Great Plains

Middle Rockies

Geologic Districts
Clark Fork Basin
Figure 2

Creek   Drummond

Silver Bow

Butte

Silver Bow

Resistant Sedimentary
Rock
Soft Sedimentary Rock
Tertiary Alluvium
Volcanic Rock
Granite

N

| 0 | 10 | 20 | 30 |
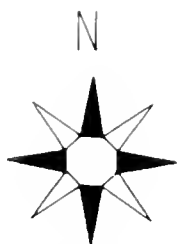
Miles

Scale 1:750,000

Landtype Associations.--These are identified by the dominant geomorphic processes responsible for shaping the landscape and influencing its functional character (Lotspeich and Platts 1982). Glacial, fluvial, alluvial, and lacustrine processes have shaped landscapes and continue to influence the manner in which water and sediments move through ecosystems. Landtype associations are subsets of geologic districts. Landtype associations seldom change in response to cultural practices, include both uplands and the valley-bottom, and are 10s to 100s of square miles in size. We used 1:250,000 scale topographic maps and aerial photos, coupled with aerial and ground reconnaissance, to identify landtype associations.

We found three landtype associations in the Clark Fork Basin that corresponded closely with geologic districts (Figure 3). Fluvial lands formed the Clark Fork River in the meta-sedimentary and volcanic geologic districts, alluvial lands formed the river in the sedimentary and tertiary fill geologic districts, and glacial lands formed the headwaters of tributaries but did not extend to the Clark Fork River.

Landtypes.--Subsets of landtype associations are identified by form and position in the landscape (Lotspeich and Platts 1982). They correlate with soil complexes, associations of potential vegetation, and areas of similar hydrologic character (Wertz and Arnold 1972). Landtypes seldom change into other landtypes in response to cultural practices other than large-scale surface mining. We used aerial photos to identify landtypes. Because our interest was with the riverine-riparian habitat complex, we identified only the valley-bottom landtype.

Valley-Bottom Types.--These are subsets of the valley-bottom landtype within landtype associations. They are distinguished by the geomorphic processes that shape the landscape and are usually distributed in a predictable manner. Valley-bottom types correspond with distinctive hydrologic characteristics, especially the relationships between stream and alluvial ground water. Valley-bottom types do not change to another type in

Landtype Associations
Clark Fork River
Figure 3

Legend:
- Glacial Lands
- Fluvial Lands
- Alluvial Lands

N

Scale 1:750,000

Miles

0   10   20   30
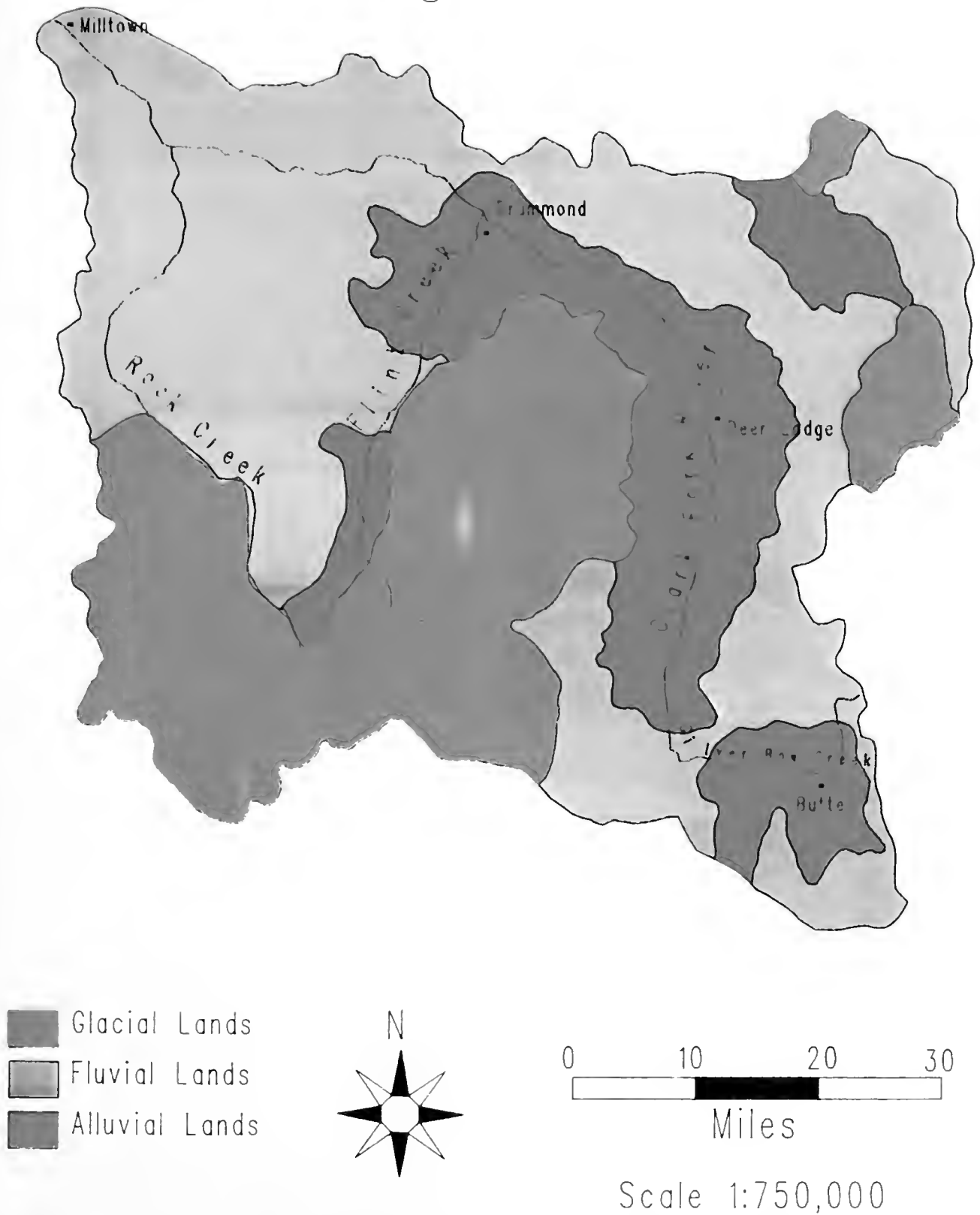
response to cultural practices other than large-scale mining. They denote areas of distinctive ecological potential. We used aerial photos and ground reconnaissance to identify valley-bottom types. We identified seven valley-bottom types along the test streams: meta-sedimentary canyon, soft sedimentary valley, soft sedimentary canyon, alluvial basin, volcanic canyon, granite valley, and granite basin.

State Types.--These describe the present condition of the stream and its banks. Natural streams change state types in response to geo-climatic conditions. An artificially stressed stream can change states through both natural and artificial processes. In the Clark Fork River, mining, road construction, railroads, urban development, grazing, agriculture, diversions, channelization, dredging, stream relocation, and logging have influenced stream states. We used 1:8,000 scale aerial photos and ground reconnaissance to identify eleven different state types in test and reference streams (Table 2).

Following the classification system from ecoregion down to valley-bottom type, we partitioned the Clark Fork River and Silver Bow Creek into discrete segments based on ecologic, geologic, and geomorphic characteristics. We further divided those segments into reaches at confluences of major tributaries. We divided a segment into reaches at a confluence if the tributary contributed more than 25% of the mean annual discharge. Thus, ecologic, geologic, geomorphic, and hydrologic characteristics defined each reach. We then divided the reaches into discrete state types. This classification system resulted in 39 state-reach segments in the test streams (Table 3).

Using the techniques described above, we identified reference stream sections that had ecologic, geologic, geomorphic, hydrologic, and state type characteristics similar to those that we identified in test streams (Appendix A). In a few cases we were unable to find state types in reference streams that matched perfectly those in test reaches. In those cases we selected state types in reference streams that matched as closely as

Table 2.--Description and codes of stream state types that we identified in test and reference streams. State types were identified from aerial and ground reconnaissance and 1:8,000 scale aerial photographs.

Code	State type	Description of state type
1	Natural	Banks in straight reaches are stable and overhanging; point bars are vegetated; cut banks are vegetated, stable, and usually overhanging.
2	Eroded banks	Banks in straight reaches are mostly eroded and unstable; point bars are mostly vegetated; cut banks are eroded and nearly vertical; bankful width is less than twice baseflow width at bends.
3	Laid-back banks	Banks in straight reaches are eroded; point-bars are mostly nonvegetated; point bars may be cut off during high flows; cut banks are eroded or laid-back; bank-full width is greater than twice base-flow width at bends.
4	Channelized	Stream sinuosity has been diminished to protect roads, railroads, urban development, industrial facilities, and other man-made features.
5	Cut-off point bars	Laid-back point bars have been cut-off forming backwaters and islands at base flow.
6	Mine tailings	Mine tailings are the dominant substrate and bank-forming material; most tailings are not vegetated.
7	Braided	Braided channels result from deposition of sediments (channel aggradation).
8	Impounded	Tailwaters of reservoirs.
9	Multiple channel	Multiple channels result from erosion of substrates.
10	Straight	Similar to channelized but not confined by man-made features; natural features restrict lateral movement of the channel.
11	Entrenched	Channel has cut the base of high terraces or residual slopes on at least one bank; high banks are unstable and constitute sediment sources to the stream.

Table 3.--Location in river miles and area in hectares of state types and reaches in the Clark Fork River and Silver Bow Creek. River mile 0 is at Milltown Dam.

Reach	State type (code)	River miles	Area (Ha)
1	Impounded (8)	0.00-1.06	31.01
	Braided/laid-back banks (7/3)***	1.06-3.52	24.40
	Channelized/cut-off point bars (4/5)***	3.52-7.30	36.52
	Laid-back banks/cut-off point bars (3/5)***	7.30-16.69	67.71
2	Channelized (4)	16.69-21.73	31.99
	Laid-back banks (3)***	21.73-23.18	9.60
	Channelized (4)***	23.18-43.41	130.12
	Eroded banks/laid-back banks (2/3)***	43.41-44.77	12.24
	Channelized (4)	44.77-47.16	17.60
3	Channelized (4)***	47.16-51.25	28.81
	Laid-back banks (3)***	51.25-51.80	3.56
4	Laid-back banks (3)***	51.80-66.55	85.33
	Channelized (4)	66.55-67.03	2.75
	Laid-back banks (3)	67.03-68.82	10.35
	Channelized (4)***	68.82-70.43	11.31
5	Channelized (4)***	70.43-79.20	49.21

***States selected for fisheries work.

Table 3.--Continued.

Reach	State type (code)	River miles	Area (Ha)
6	Eroded banks (2)***	79.20-81.93	11.53
	Channelized (4)	81.93-84.03	9.19
	Laid-back banks/channelized (3/4)	84.03-88.71	21.41
	Eroded banks (2)	88.71-93.68	21.48
	Channelized (4)***	93.68-96.20	11.19
	Entrenched (11)	96.20-97.76	5.96
	Eroded banks (2)	97.76-100.67	14.02
	Entrenched (11)	100.67-101.87	5.41
	Eroded banks (2)***	101.87-107.35	23.25
	Natural/eroded banks (1/2)***	107.35-115.25	25.22
7	Mine tailings (6)	115.25-119.09	5.27
	Eroded banks (2)***	119.09-120.18	1.50
	Channelized/mine tailings (4/6)	120.18-120.60	0.57
	Mine tailings (6)	120.60-123.46	3.70
	Channelized/mine tailings (4/6)***	123.46-126.94	4.50
	Mine tailings (6)	126.94-127.39	0.58
8	Mine tailings (6)	127.39-128.21	0.84
	Channelized/mine tailings (4/6)	128.21-128.70	0.50
9	Channelized/mine tailings (4/6)***	128.70-132.00	3.37

***States selected for fisheries work.

Table 3.--Concluded.

Reach	State type (code)	River miles	Area (Ha)
10	Channelized/mine tailings (4/6)	132.00-133.36	2.21
	Laid-back banks/mine tailings (3/6)***	133.36-136.95	5.85
	Channelized/mine tailings (4/6)	136.95-143.47	10.62
	Channelized (4)	143.47-145.09	2.64

***States selected for fisheries work.

possible the state types in test streams. For example, we matched a straight state in a reference reach with a channelized state in a test reach if there was no channelized state in the reference stream. The only difference between a channelized state and a straight state is that sinuosity in the channelized state is reduced by man-made features, whereas natural features restrict sinuosity in straight states (Table 2).

Selection of Sampling Sites.

After we classified the Clark Fork River and Silver Bow Creek into distinct reaches and state types, we selected state types within each reach for fisheries sampling (Table 3). In most cases we selected randomly one of each state type within each reach for fisheries work. We did not select the impounded state in reach 1 or the entrenched and laid-back bank/channelized states in reach 6 as they constituted only 7% of their respective reaches. We randomly selected two eroded bank states in reach 6 because that state type made up 71% of the reach. We did not select state types in reach 8 because it was a small reach with ecologic, geologic, and geomorphic, characteristics similar to those in reach 9. We ended with 19 state-reach segments in the test streams.

We matched each of those with a corresponding reference segment (Table 4).

After selecting test and control states, we divided them into 100-m (328 ft) long units. We used an episometer on the most recent USGS 7.5 minute series topographic maps to measure lengths of stream states. We measured state lengths from downstream to upstream. A most upstream unit less than 100-m long was not considered for sampling. In each state type we selected randomly four 100-m long units to serve as sampling sites for fish abundance and biomass. We selected one of the four sites in each state type for microhabitat studies and physical habitat simulation (PHABSIM) work. When possible, we selected those sites randomly, but some sites were selected based on accessibility.

In a few cases we matched one reference state type with two similar test state types (Table 4). For example, we matched the two eroded bank states in reach 6 with one eroded bank state in Flint Creek, and the channelized states in reaches 4 and 5 with a straight state in the Big Hole River. In those situations, we randomly chose eight 100-m long sampling sites from the reference state: four were matched randomly with one test state and the rest corresponded with the other test state. We used only one of the eight sites for microhabitat and PHABSIM work.

Microhabitat.

We define microhabitat as discrete stream variables that collectively create site-specific aquatic habitat for fish. Here our purpose was to assess habitat differences between test and reference sites at a resolution greater than state type. We used that information to standardize population estimates.

From July to October, 1991, we described microhabitat within test and reference sites with a stream cross-sectional method outlined by Platts et al. (1983). We delineated

Table 4.--Matching of state types in test and reference streams that were sampled for fish and habitat data. For reference streams, river mile 0 is at the stream's confluence. We distinguish between the downstream and upstream eroded-bank states in Reach 6 with the codes 2d and 2u, respectively.

Test Stream			Reference Stream			
Stream	Reach	State type (code)	Reach	State type (code)	Rm	
Clark Fk	1	Braided/Laid-back (7/3)	1	Laid-back-banks (3)***	0.00-1.20	
Clark Fk	1	Channelized/Cut-off-bars (4/5)	1	Laid-back-banks (3)***	3.20-5.09	
Clark Fk	1	Laid-back/Cut-off-bars (3/5)	1	Laid-back-banks (3)	0.00-1.20	
Clark Fk	2	Laid-back-banks (3)	1	Laid-back-banks (3)	3.20-5.09	
Clark Fk	2	Channelized (4)	1	Straight (10)	5.09-10.40	
Clark Fk	2	Eroded-banks (2)	1	Eroded-banks (2)	1.20-3.30	
Clark Fk	3	Channelized (4)	5	Straight (10)***	29.47-30.73	
Clark Fk	3	Laid-back-banks (3)	5	Laid-back-banks (3)	28.06-29.47	
Clark Fk	4	Laid-back-banks (3)	5	Laid-back/Multiple (3/9)	17.82-28.06	
Clark Fk	4	Channelized (4)	5	Straight (10)	29.47-30.73	
Clark Fk	5	Channelized (4)	4	Straight (10)	15.37-17.82	
Clark Fk	6	Eroded-banks (2d)	1	Eroded-banks (2)***	7.84-10.59	
Clark Fk	6	Channelized (4)	1	Channelized (4)	38.89-44.01	
Clark Fk	6	Eroded-banks (2u)	1	Eroded-banks (2)	7.84-10.59	
Clark Fk	6	Natural/Eroded-bk (1/2)	2	Natural/Eroded-bk (1/2)	51.58-53.55	
Silver Bow	7	Eroded-banks (2)	3	Eroded-banks (2)	50.72-73.00	
Silver Bow	7	Chan/Laid-bk/Tailings (4/6)	1	Laid-back/Multiple (3/9)	2.02-11.53	
Silver Bow	9	Chan/Laid-bk/Tailings (4/6)	1	Laid-back/Eroded-banks (3/2)	0.00-5.23	
Silver Bow	10	Laid-back/Tailings (3/6)	3	Laid-back/Eroded-banks (3/2)	8.82-22.49	

***Reference states that were matched with two similar test states.

each transect by stretching a measuring tape across the upstream end of each site. The transect passed through a reference point defined as the channel mid-point. The transect line extended from that point and traversed across the stream perpendicular to the main streamflow to establish reference points on the right and left banks. We defined the right bank by facing downstream. We determined the next transect line by measuring along the middle of the channel 11 ft downstream from the previous mid-point. This measurement determined the position of the second transect line and reference point on the right bank. We repeated this process until we established 30 transects.

We measured the following habitat variables at each transect line:

Channel width.--This is the distance along the transect line beginning at the high water mark on one bank and ending at the high water mark on the opposite bank. We recorded channel width to the nearest foot.

Wetted perimeter width.--This is the distance from the edge of the water on one bank to the edge on the opposite bank. We recorded wetted width to the nearest foot.

Riffle width.--These are portions of the water column identified by fast water velocity, relatively shallow stream depths, and a relatively steep water surface slope with a straight to convex channel profile (Helm 1985). We recorded the width of riffles that occurred on the transect line to the nearest foot.

Run width.--This is the area of the water column that does not form distinguishable pools or riffles, but has a rapid nonturbulent flow (Helm 1985). Runs are too deep to be a riffle and too fast to be a pool. They have uniform, flat channel profiles. We measured the width of runs that occurred on the transect line to the nearest foot.

Pool width.--These are deeper than riffles or runs and occur in an area of the water column that has slow water velocities (Helm 1985). The streambed slope of the pool often approaches zero and has a concave shape. Pools can contain large eddies

with widely varying directions of flow compared to the downstream flow in riffles and runs. We measured the width of pools that occurred on the transect line to the nearest foot.

Pool rating.--This rating estimates the capability of the pool to provide fish survival and growth requirements (Platts et al. 1983). This requires the combination of a cover analysis with direct measurements of the greatest pool diameter and depth. Materials or conditions such as logs, organic debris, overhanging vegetation, boulders, undercut banks, or water depth provide pool cover and protection for fish. If the transect line intercepted more than one pool, we summed the pool widths times their respective quality ratings (ratings are given in Platts et al. 1983). We divided this total by the pool total width to estimate the weighted average pool rating. To avoid inconsistencies, the same observer described pool ratings at all transects.

Bank angle.--This measurement monitors land uses that change the morphology and location of the streambanks. We used a clinometer to measure the angle formed by the downward sloping streambank as it meets the more horizontal stream bottom. For undercut banks, we determined bank angle by placing the clinometer on the top of a measuring rod as it formed the angle determined by the protruding edge of the bank to the mid-point of the undercut under the transect line (Platts et al. 1983). If the bank was not undercut, we measured bank angle by placing the clinometer on the top of the measuring rod that was aligned parallel to the streambank along the transect. Here we subtracted the clinometer reading from 180° to get the bank angle. For each transect, we reported the mean of the two bank angles.

Average and thalweg depths.--We determined stream depth across a transect by averaging water depths taken at three locations: one-fourth, one-half, and three-fourths the stream width. We then divided the total of the three water measurements by four to account for the zero depths at the stream margin where the water surface and the bank

meet. We recorded thalweg depth as the deepest point along the transect. That was the largest value we found after taking several measurements in the deepest portions of the channel under the transect line. We measured depths to the nearest foot.

Substrate.--We measured the composition of the channel substrate at one-foot increments along the transect line. At each increment, we visually estimated the substrate composition of the stream bottom and assigned bottom materials to substrate classes (i.e., boulder, rubble, gravel, and fines) described by Platts et al. (1983). We then totaled the individual one-foot classes of substrate to get the amount of streambed in each of the size classes. The combined substrate widths, measured to the nearest foot, equalled the total transect width.

Bank cover.--Following Platts et al. (1983), we rated the ability of vegetation and other materials on the streambank to resist erosion from flowing water. The rating corresponded primarily to stability generated by the vegetative cover except in those cases where bedrock or rubble stabilized the bank. One observer rated both streambanks at each transect line to keep bias consistent. For each transect we reported the mean of the two bank cover ratings.

Vegetative overhang.--This rated only the vegetation overhanging the water column within 12 inches of the water surface (Platts et al. 1987). We measured vegetative overhang as the distance between the point of the farthest protrusion of the streambank over the water surface to the farthest point that vegetation covered the water column. This measurement did not include undercut banks. We measured overhang to the nearest inch and reported the mean of the two measurements at each transect.

Canopy cover.--This is a measure of the amount of shading a stream receives from canopy formed by trees and shrubs that hang over the stream at a distance greater than 12 inches above the stream surface. We measured canopy cover as the percentage of a transect line covered overhead by trees and shrubs.

Bank alteration .--This reflects changes that take place in the bank from any force (Platts et al.1983). The rating consisted of five classes. Each, except the one with no alteration, had an evaluation spread of 25 percentage points (Platts et al. 1983). To assess bank alteration, we first assigned the bank to one of the five classes and then described the actual percent of instability. The same observer described bank alteration at each transect. For each transect we reported the mean of the two ratings.

Woody debris .--Woody debris consisted of submerged logs, root wads, and brush in the stream channel. We measured the amount of woody debris along a transect line to the nearest foot and then calculated it as a percentage of the total stream width.

Sun arc.--We used the angle of the sun arc as an index of solar radiation input. Materials that intercept the sun's rays (e.g., streamside vegetation, logs, debris, bridges, trees, high streambanks, and narrow canyons) reduce the degrees of the arc. We used a clinometer to measure the angle made by the arc of the sun at the mid-point of the transect. We measured the angle from the channel horizontal to the sun horizon on each side of the mid-point along the transect. We then added those two angles together and subtracted that sum from 180° to get the sun arc degrees for that transect. For uniformity, we used the sun's arc on 1 August for all measurements (Platts et al. 1983).

Bank undercut .--Undercut is an indicator of how successfully streambanks are protected under alternative land uses (Platts et al. 1983). We measured the undercut, if it existed, under the transect line as the distance from the farthest point of protrusion of the bank to the farthest undercut of the bank. Water level did not influence this reading. We measured undercuts to the nearest inch and recorded the mean of the two measurements at each transect.

The multivariate Hotelling-Lawley trace statistic assessed microhabitat differences between test and reference sites. We removed from the multivariate analyses microhabitat variables that were correlated ($r \geq 0.80$) with other variables or had zero

variances in both the test and reference sites. Because habitat differed significantly between the 19 pairs of test and reference sites (Appendix B), we analyzed each microhabitat variable independently to find which variables contributed to the overall significance of the multivariate test. Whenever possible we used the methods described in Miller (1986) to calculate 99% confidence intervals around the ratio of means for each microhabitat variable. We considered the difference in a microhabitat variable between test and reference sites significant statistically if the number "1" did not fall within the confidence interval for the ratio. For variables with a mean of zero in either the test or reference site, a one-sample t-test assessed if the non-zero mean differed significantly from zero. In those cases where the means of a variable were not zero and we could not calculate a confidence interval for the ratio, a two-sample t-test assessed differences between means. To reduce the Type I error rate we used alpha = 0.01.

Physical Habitat Simulation.

Microhabitat differed significantly between test and reference sites (Appendix B). Consistent among the 19 pairs was the significant difference in channel widths, habitat type widths, depths, and substrates between test and reference sites (Appendix B). The physical habitat simulation (PHABSIM) system developed by the U.S. Fish and Wildlife Service (Milhous et al. 1984) uses those variables to assess potential fish habitat. That is, PHABSIM uses measurements of depth, velocity, substrate, and cover to quantify fish habitat available at a given flow. PHABSIM is used generally to predict the effects of changes in flow regime on fish habitat within a given reach of stream, but here we used it to assess habitat availability in test and reference sites. We used the PHABSIM output, which is in the form of weighted usable area (WUA) in mid-August, to standardize population estimates in test and reference sites. Thus, our population estimates in test and reference sites could be compared without the confounding effects

of habitat and flow differences.

Following Trihey and Wegner (1981), we collected standard PHABSIM measurements along four transects spaced at 23 m (105 ft) intervals within each habitat sampling site. We visited each site three times between July and October, 1991. During the first visit we recorded water velocities, depths, and substrate composition at 20 to 30 points along each transect, and recorded water surface elevations. On subsequent visits we measured discharge at one or two transects and recorded water surface elevations. We measured water velocities with a Swoffer Instruments velocity meter (accuracy $\pm$ 1%) and a Marsh-McBirney electromagnetic velocity meter (accuracy $\pm$ 2%). We based substrate composition on a modified particle scale in which codes ranged from 1 for mud and silt to 9 for bedrock (Table 5). We generated a three-digit numeric score (xx.x) that weighted the percentages of the two dominant substrates. The first two digits represented the dominant and subdominant substrates, respectively; the third expressed the percentage of the dominant material. Thus, we coded a cell that contained 10% sand (code 2), 60% medium gravel (code 4), and 30% small cobble (code 6) as 46.6.

We loaded the hydraulic and channel morphology data into the single-flow version of the IFG-4 program (Milhous et al. 1989). We executed the PHABSIM with depth, velocity, and substrate curves for adult (>8 in), juvenile (2-8 in), and fry (<2 in) brown trout (Raleigh et al. 1986), rainbow trout (Raleigh et al. 1984), and brook trout (Bovee 1978; Hanson et al. 1987). We used the HABTAT program to convert the hydraulic output to WUA (Milhous et al. 1989). Standard output of WUA is in ft^2/1000 linear feet. To describe the WUA in our 100-m long sites, we divided the WUA output by 3.049 to get WUA in ft^2/100 linear meters. We then multiplied that quotient by 0.0929 to get WUA in m^2/site. Because we assumed that summer low flow was a major factor regulating populations in test and reference sites, we compared WUA between test and reference sites at the lowest flows that we recorded (Table 6).

Table 5.--Substrate codes for stream channel materials by particle size.

Code	Particle diameter size (inches)	Sediment classification
1	<0.001	mud and silt
2	0.001-0.1	sand
3	0.1-0.5	small gravel
4	0.5-1.5	medium gravel
5	1.5-3.0	large gravel
6	3.0-6.0	small cobble
7	6.0-12.0	large cobble
8	> 12.0	boulder
9		bedrock

Population Estimates.

We estimated densities of trout by direct underwater observation and electrofishing within test and reference sites during the period July through October, 1991 and September, 1992. Underwater observation by snorkeling is a quick, inexpensive, and nondestructive census method that is not limited by deep, clear, nonconductive water as is electrofishing. Several studies (e.g., Schill and Griffith 1984; Hicks and Watson 1985; Zubik and Fraley 1988; Hillman et al. 1992) have shown that this is an unbiased census technique, however, Hillman et al. (1992) showed that it may be biased when stream temperatures fall below 14°C and concealment cover is abundant. Under those conditions electrofishing generally works well.

We used underwater observation to estimate populations in a site if the water was warmer than 14°C, underwater visibility was at least 1.5 m, and a two-inch fish on the

Table 6.--Range of stream flows (cfs) in test and reference sites that were used to assess weighted usable area. Stream flows were measured three different times between July and October, 1991.

| | Test stream | | | Stream | | Reference stream | | |
Stream	Reach	State code	Flow range		Reach	State code	Flow range
Clark Fk	1	7/3	638-825	Rock Ck	1	3	114-473
Clark Fk	1	4/5	502-820	Rock Ck	1	3	178-468
Clark Fk	1	3/5	535-817	Rock Ck	1	3	114-473
Clark Fk	2	3	253-417	Rock Ck	1	3	178-468
Clark Fk	2	4	238-423	Rock Ck	1	10	152-534
Clark Fk	2	2	222-358	Rock Ck	1	2	176-539
Clark Fk	3	4	179-371	Big Hole	5	10	276-344
Clark Fk	3	3	210-396	Big Hole	5	3	258-279
Clark Fk	4	3	120-223	Big Hole	5	3/9	246-291
Clark Fk	4	4	144-254	Big Hole	5	10	276-344
Clark Fk	5	4	151-222	Big Hole	4	10	240-305
Clark Fk	6	2d	80-157	Flint Ck	1	2	15-80
Clark Fk	6	4	28-147	Ruby	1	4	9-44
Clark Fk	6	2u	16-102	Flint Ck	1	2	15-80
Clark Fk	6	1/2	24-53	Beaverhead	2	1/2	72-190
Silver Bow	7	2	32	Ruby	3	2	94-105
Silver Bow	7	4/6	33-39	Big Hole	1	3/9	68-261
Silver Bow	9	4/6	21-29	Bison Ck	1	3/2	4-12
Silver Bow	10	3/6	23-32	Bison Ck	3	3/2	0-3*

*Water velocities were too low to accurately estimate flows.

streambottom could be identified clearly. We snorkeled on clear days between 0900 and 1600 hours.

During snorkel surveys a team of two to five observers estimated trout and whitefish numbers in sampling sites. Two observers had over 4,000 hours of snorkeling experience, one had over 1,000 hours, and the other two had under 500 hours of snorkeling experience. Observers floated downstream through the site and estimated population numbers if water depth was greater than 0.5 m (20 in); if the water depth was under 0.5 m deep, observers crawled upstream through the site. We measured site widths and lengths with an optical rangefinder.

During downstream floats, members of the team entered the water about 40 m upstream from the site. They maintained a prescribed spacing from one another by holding onto connected lengths of 3-cm-diameter plastic (PVC) pipe. Underwater visibility determined spacing, which ranged from 2.5 to 8.5 m. Members of the team counted only those fish within the site that passed underneath in a lane between themselves and the observer to their left. The flexible PVC pipe enabled the observers on each end of the counting lane to position themselves about 1 m ahead of the others. This facilitated counting any fish that tended to move laterally along the counting line. An observer moved upstream in the water along the stream margin to count fish stationed close to each bank.

For surveys conducted by crawling upstream, members of the team entered the water about 25 m downstream from the sampling site and then moved upstream at a uniformly slow rate into and through the site. Observers counted only those fish that were within the observers predetermined counting lane. Communication among observers consisted of a series of slow-motion hand signals. Thus, fish that moved across counting lanes were not counted twice.

We divided trout into 13 total length size classes, each two-inches wide, from 1 to

25 inches. For each trout that we observed, we estimated its length and assigned it to one of the size classes. We repeatedly tested the accuracy and precision of our estimates by having observers estimate lengths of dead fish or sticks of known size. Observers consistently assigned objects to the correct size class.

We used electrofishing to estimate densities of fish in sites where water clarity precluded the use of underwater observation (Reach 3 Bison Creek). We also used electrofishing to assess trout densities and biomasses in the natural stream state type with eroded banks (1/2) in reach 6 on the Clark Fork River and in its reference segment on the Beaverhead River in September, 1992. We placed 5-mm-mesh seines at the upper and lower ends of each study site. The sites were electrofished by working downstream with a backpack shocker. Using the same equipment and personnel on each pass, we made four complete passes, retaining fish from each pass in separate holding tanks. We measured (inches) and weighed (pounds) trout taken during each pass, and estimated population numbers with the maximum-likelihood formula (Van Deventer and Platts 1989). Personnel of the Montana Department of Fish, Wildlife, and Parks (MDFWP) electrofished sites in Silver Bow Creek.

We also electrofished to validate snorkel estimates and to develop length and weight relationships. We selected two sites on the Clark Fork River and one on each of the reference streams except the Ruby River. We were unable to snorkel in the Ruby River in September during the validation work because water clarity was 0.3 m. We tried to select sites that had more habitat diversity than most population sites (i.e., undercut banks, instream woody debris, overhanging vegetation, and deep pools). We believed that if density estimates from snorkeling and electrofishing were similar in sites with diverse habitat, then underwater counts in sites with less diversity should also be accurate. We used a "blind experiment" to validate snorkel estimates. First, we blocked the sites with seines and then snorkeled through the sites and estimated numbers and

sizes of trout. Immediately after we snorkeled, personnel of MDFWP electrofished the sites using the four-pass removal-depletion method described above. They measured (inches) and weighed (pounds) all trout taken during each pass, and estimated densities of trout. We then compared numbers estimated by the two independent methods and assumed that counts estimated by electrofishing represented the "true" population size. Both methods produced similar results (Appendix C). Therefore, no correction was needed to make snorkel counts comparable with electrofishing estimates.

We used length and weight measurements from trout collected by electrofishing in validation sites to develop equations to predict weights of fish observed during snorkeling. We supplemented those data with length and weight measurements collected by MDFWP biologists, who had electrofished in segments of the rivers that we worked. Because the relationship between weight and length of fish is a power function, we transformed both variables with logarithms, and then estimated the constants with simple linear regression. We developed a separate equation for each species in each stream sampled (Appendix D). We used those equations to estimate the weights of trout in sites that we snorkeled.

We standardized counts and weights of trout to number per WUA and weight per WUA (see Appendix E for WUA values for test and reference states). We multiplied numbers/WUA and weight/WUA by 1,000 so that analyses would include whole fish rather than fractions of fish. We also expressed and analyzed densities and biomasses of trout as number per hectare and weight per hectare. For analyses, we divided trout into three age categorizes: fry (<2 inches), juvenile (2-8 inches), and adult (>8 inches). For each species and age class, we calculated the mean density (ratio of mean number to WUA and mean number to mean area) and mean biomass (ratio of mean weight to WUA and mean weight to mean area) for each test and reference stream state type. We then used the sign test for two related samples to assess if mean densities and biomasses

in test states differed significantly from the mean densities and biomasses in reference states. We considered tests with probability values $P \leq 0.01$ as statistically significant.

RESULTS

Trout were significantly more abundant in reference states than in test states (Figure 4; Appendix F). On average, densities of all trout were 4.1 times more abundant in reference states than in test states[2]. Densities of trout ranged from 0.0 to 583.7 fish/ha in test states and from 19.2 to 794.1 fish/ha in reference states (Figure 4). After we removed habitat and flow effects, trout were 2.2 times more abundant in reference states than in test states, and numbered 0.0 to 1130.0 fish/WUA in test states and 7.8 to 955.7 fish/WUA in reference states (Figure 4). Total trout biomass was also greater (4.5 times) in reference states (Figure 5). Biomass in test states ranged from 0.0 to 126.9 pounds/ha and 4.2 to 271.2 pounds/ha in reference states. When we removed the effects of flow and habitat, trout biomass was on average 2.1 times greater in reference states than in test states, and ranged from 0.0 to 333.4 pound/WUA in test states and 2.7 to 300.8 pounds/WUA in reference states.

Juvenile trout (2-8 inches) made up 66% of all trout in test states and 63% of the trout in reference states. Juvenile trout were 3.9 times more abundant in the reference states, and ranged from 0.0 to 416.3 fish/ha and 11.7 to 541.9 fish/ha in test and reference states, respectively (Figure 6). With flows and habitat removed from the analyses, densities of juveniles in the reference states were 2.4 times greater than those

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[2]Differences in mean densities and biomasses represent the magnitude of the differences between mean scores in test and reference sampling sites; they have not been weighted for areas of state or reaches.

Figure 4.--Mean densities of all trout in test and reference states.
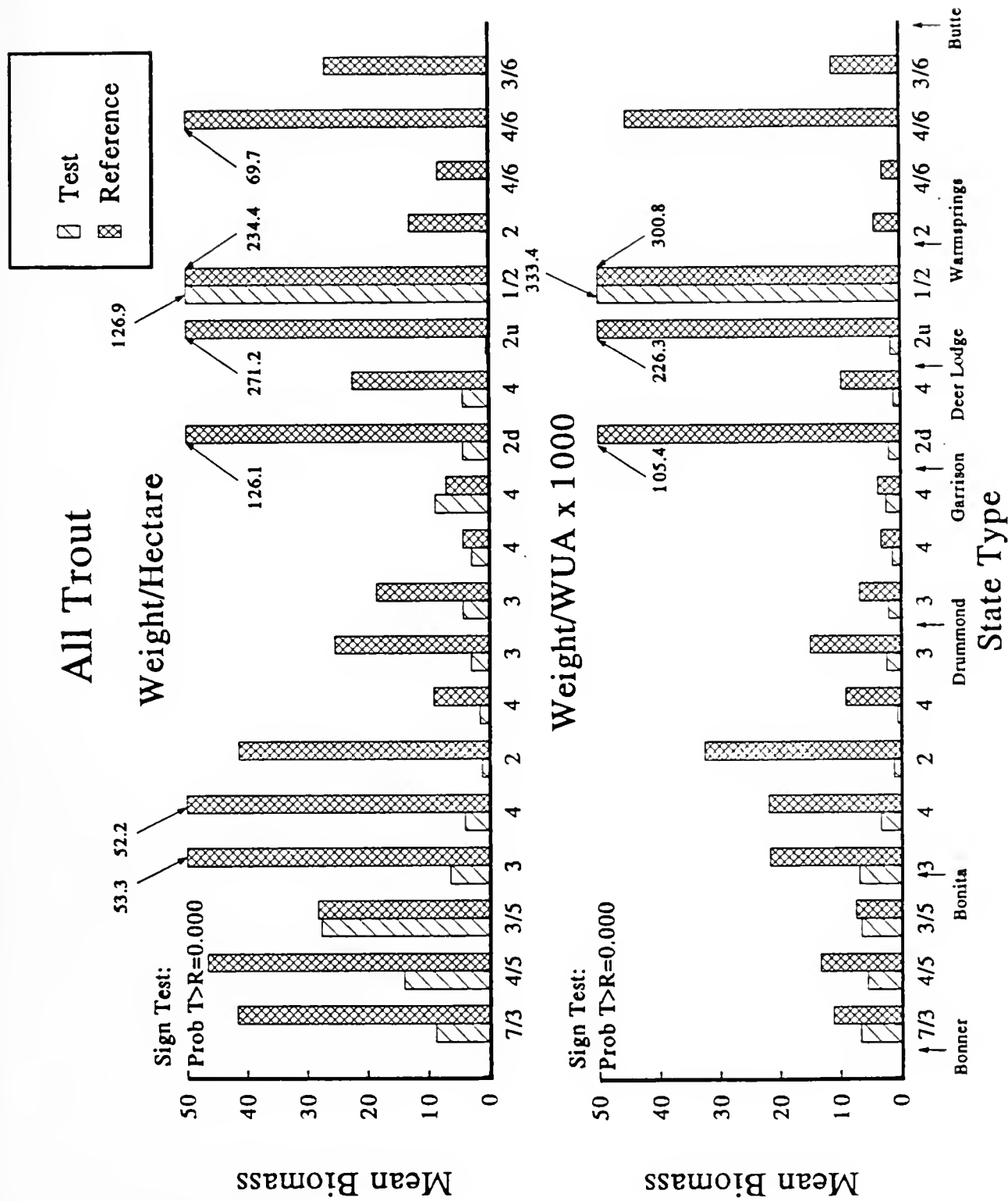
Figure 5.--Mean biomasses of all trout in test and reference states.
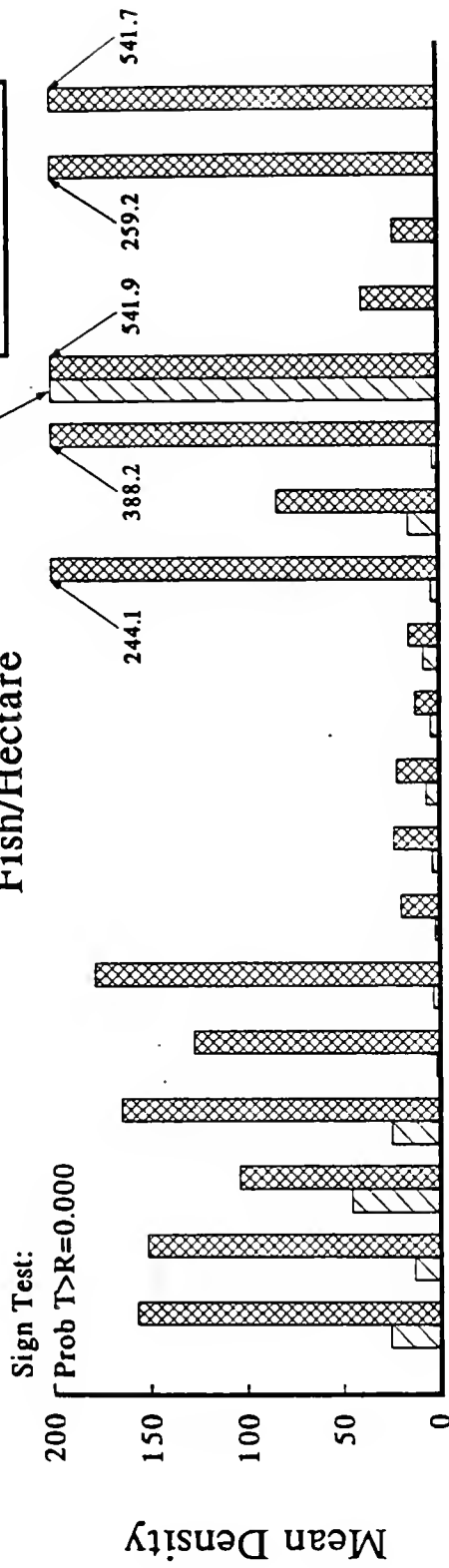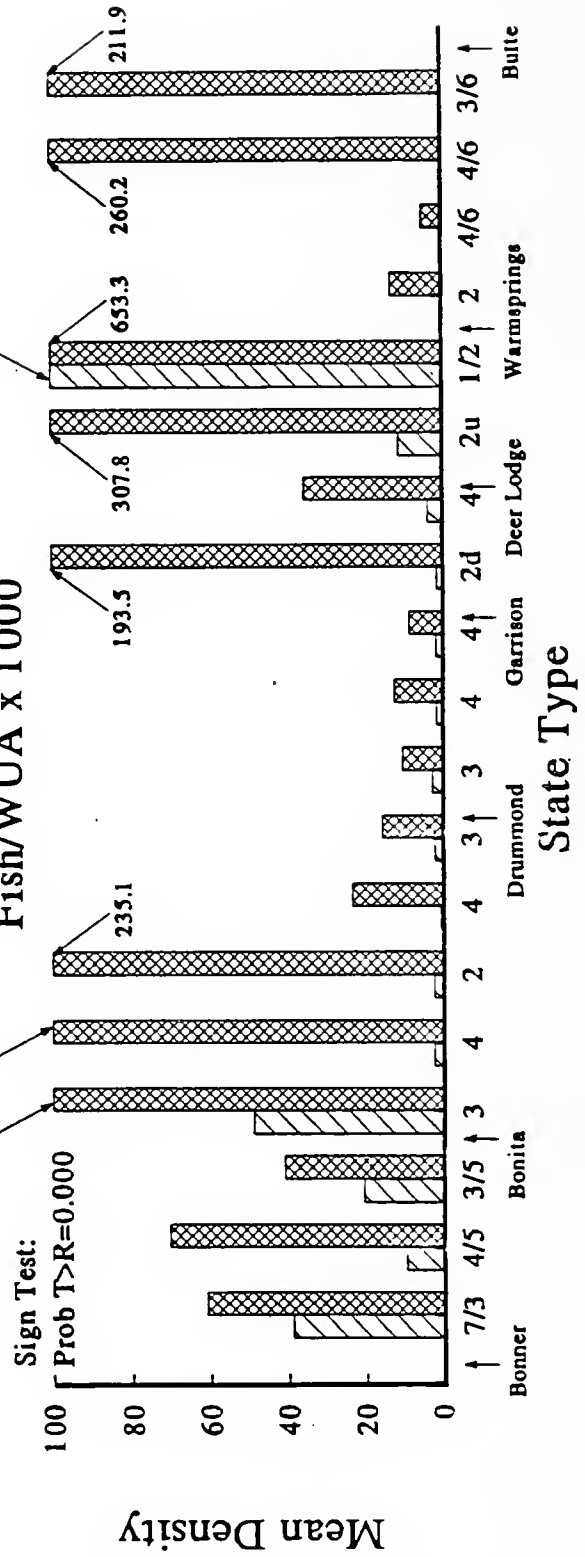
Figure 6.--Mean densities of all juvenile trout in test and reference states.

in test states. In both the test and reference states, juveniles constituted about 16% of the biomass of all trout. Biomass of juveniles was on average 3.0 times greater in reference states than in test states when we removed flow and habitat differences (Figure 7). In the Clark Fork River, juvenile trout were most abundant in Reaches 1, 2, and 6 (Figure 6). We found few juvenile trout in Reaches 3, 4, and 5. We found no juvenile trout in Silver Bow Creek.

Adult trout (>8 inches) were significantly more abundant in reference states than in test states (Figure 8). In terms of fish/ha, trout were on average 4.5 times more abundant in reference states than in test states, and 1.9 times more abundant in reference states than in test states after we removed flow and habitat effects. With habitat and flow effects removed, trout in reference sites ranged from 3.1 to 334.7 adults/WUA, while in test states they ranged from 0.0 to 467.4 adults/WUA. Biomass of adult trout was also significantly greater in reference sites than in test sites (Figure 9). When we removed habitat and flow differences, biomass of adult trout was 2.0 times greater in the reference states than in the test states. As with juveniles, adult trout were most abundant in Reaches 1 and 6, with few adults in the other reaches. We found no adult trout in Silver Bow Creek.

Brown Trout.

Brown trout were significantly more abundant in reference states than in test states. On average their densities and biomasses were respectively 3.7 times and 4.8 times greater in reference states than in test states (Figure 10 & 11). After we removed flow and habitat effects, brown trout were 1.8 times more abundant in reference states than in test states. Brown trout weight per WUA ranged from 0.0 to 330.9 pounds in test states and 0.0 to 297.9 pounds in reference states (Figure 11).

Juvenile brown trout made up 69% and 61% of all brown trout in the respective

Figure 7.--Mean biomasses of all juvenile trout in test and reference states.
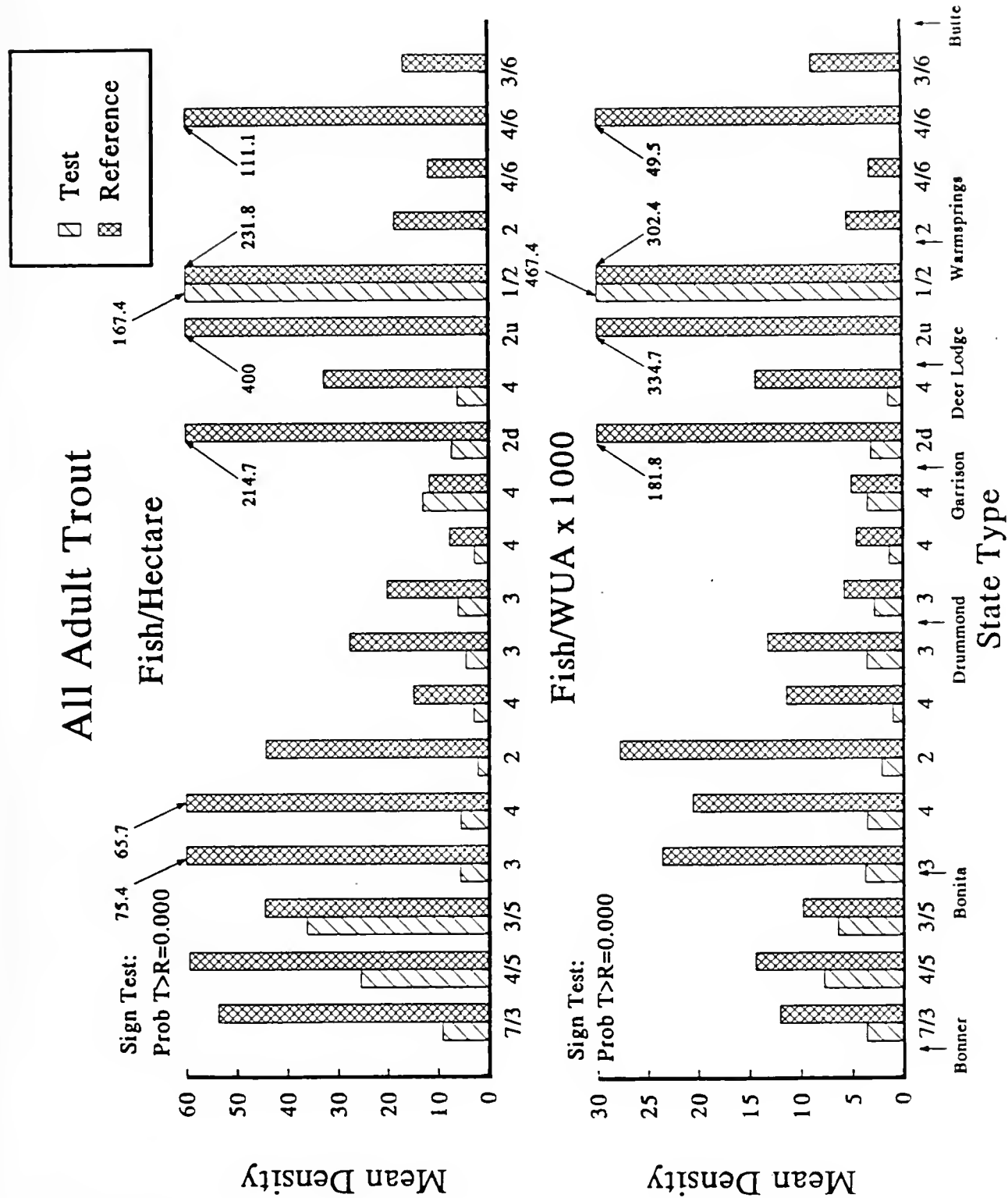
Figure 8.--Mean densities of all adult trout in test and reference states.
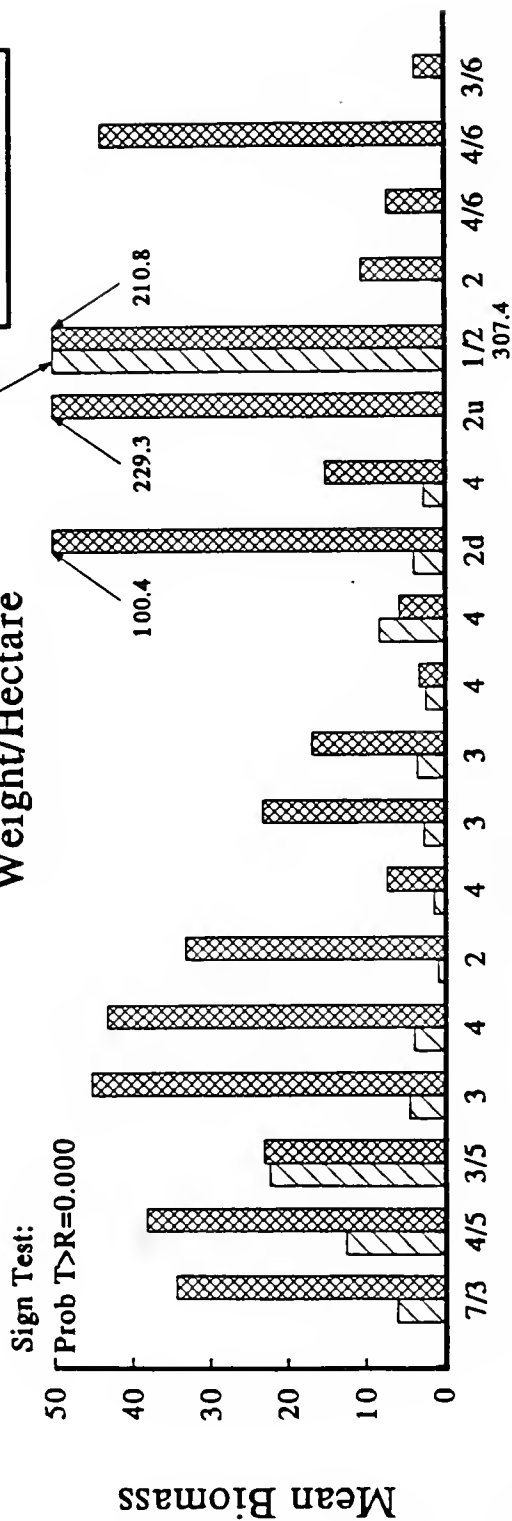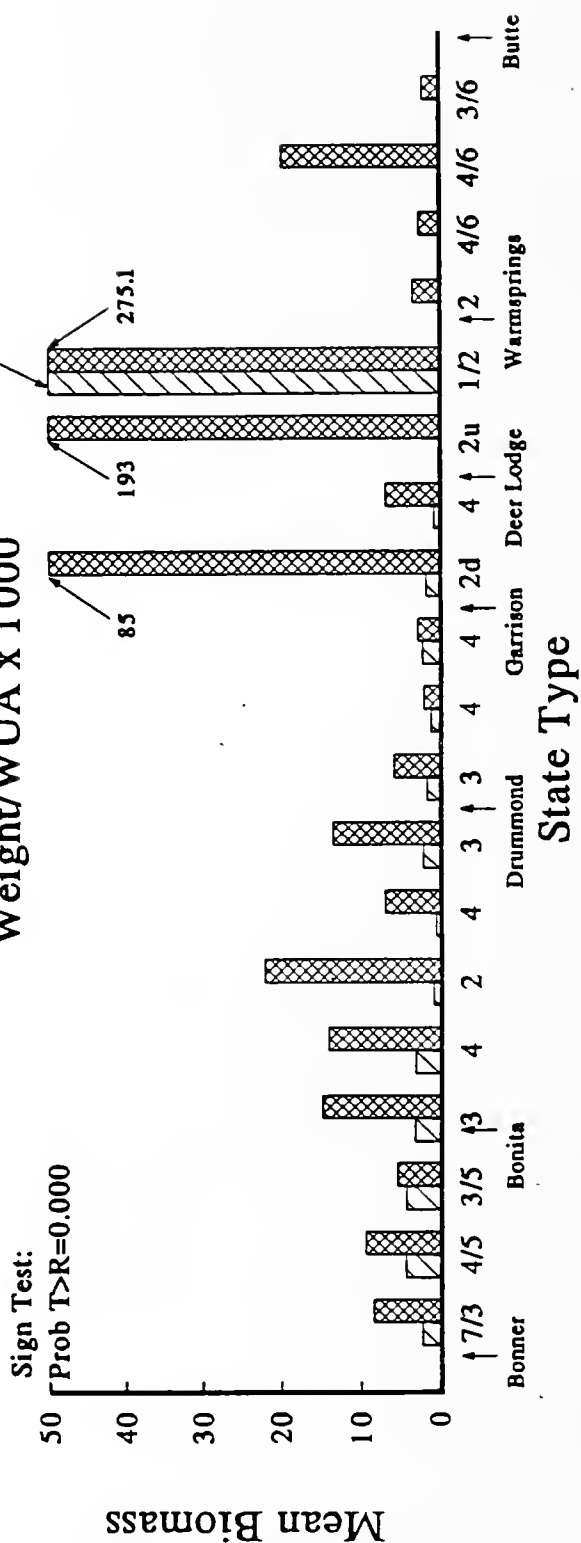
Figure 9.--Mean biomasses of all adult trout in test and reference states.
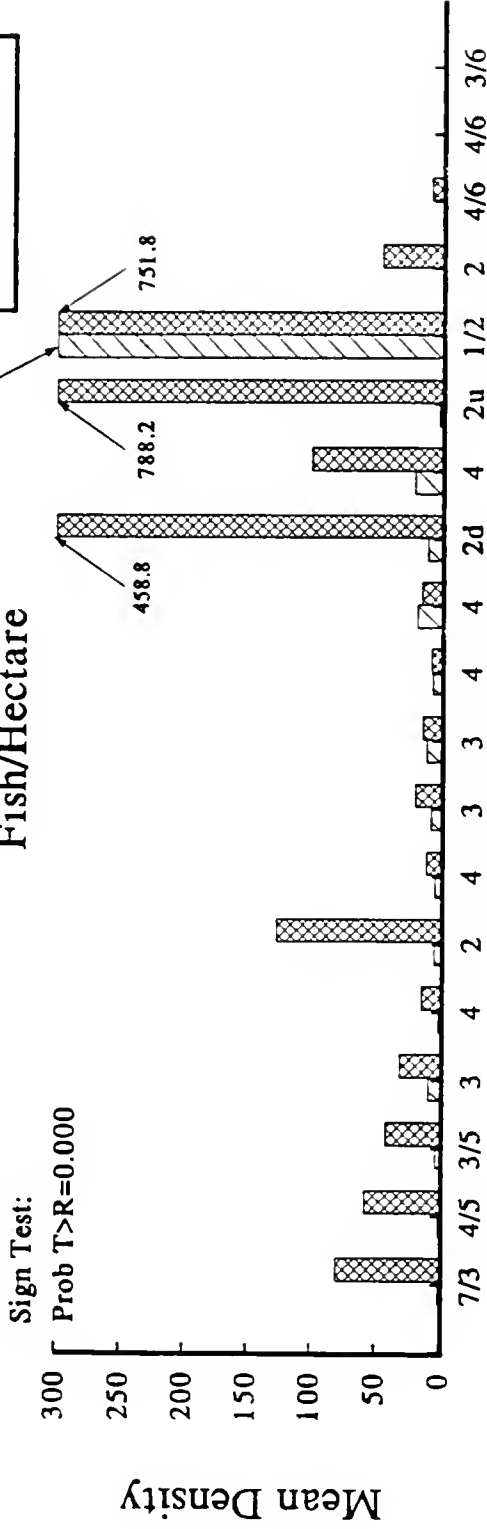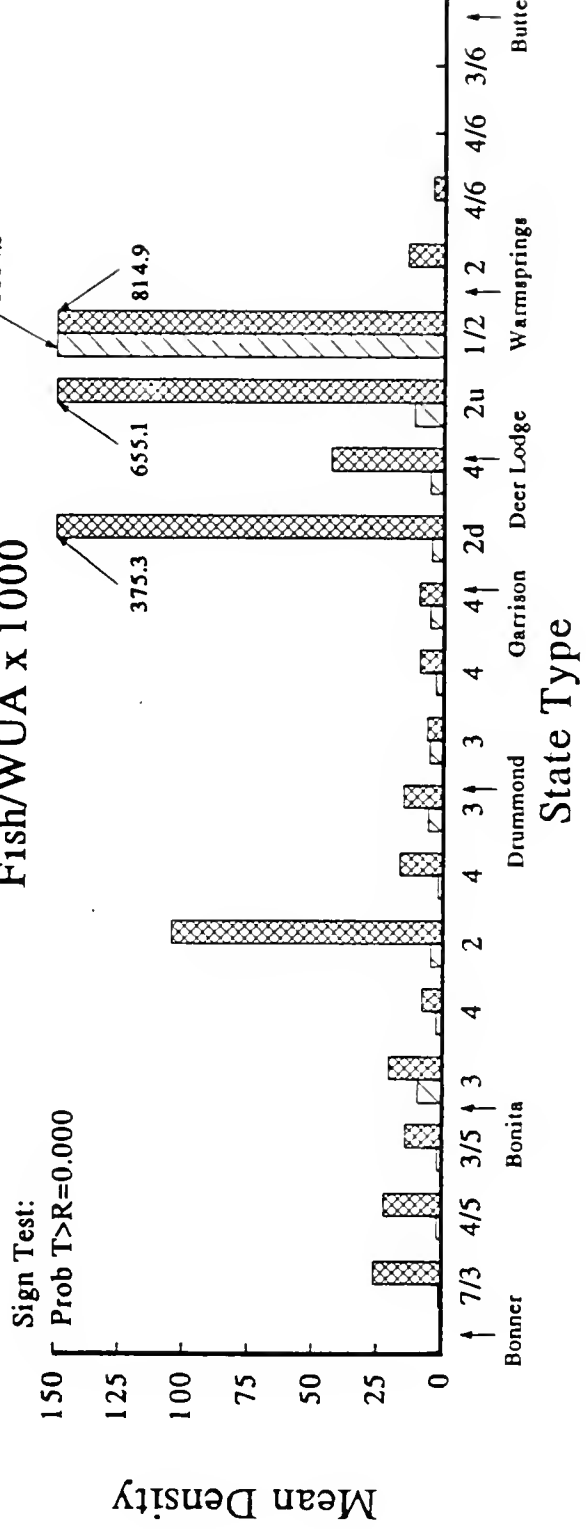
Figure 10.--Mean densities of all brown trout in test and reference states.
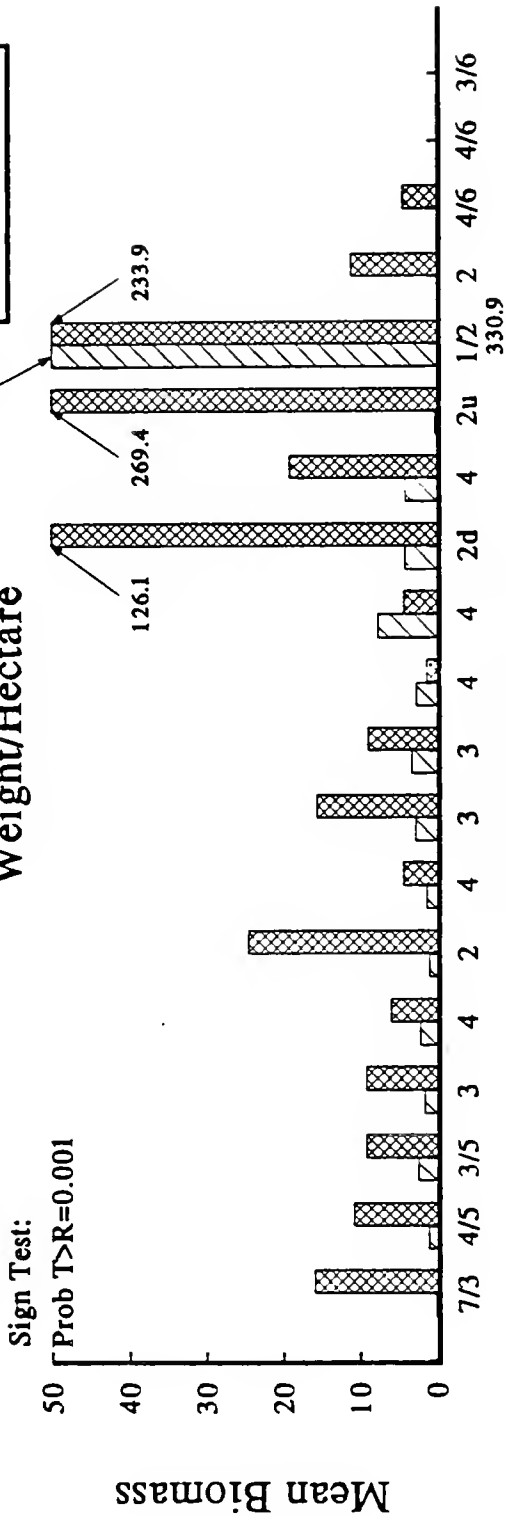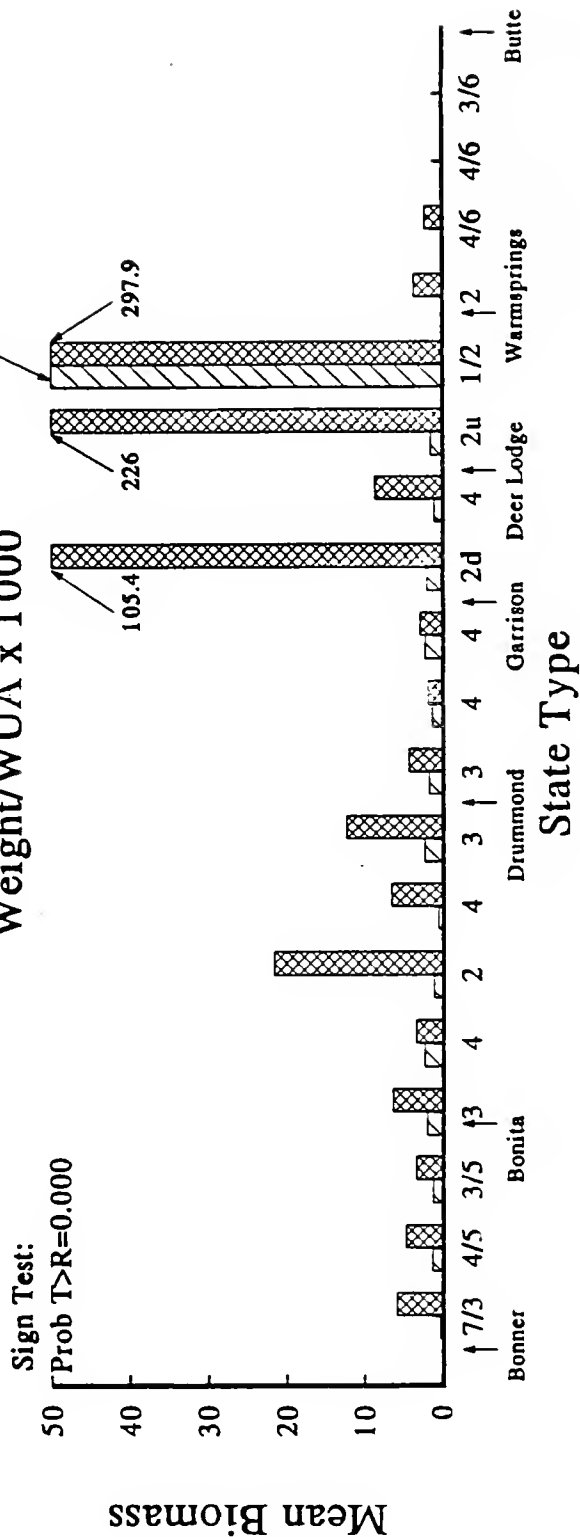
Figure 11.--Mean biomasses of all brown trout in test and reference states.

test and reference states. When we removed flow and habitat differences, juvenile brown trout were on average 1.8 times more abundant in reference states than in test states (Figure 12). Biomass of juvenile brown trout was also significantly greater in the reference states (Figure 13). Juveniles had 2.9 times more biomass in reference states than in test states after we removed habitat differences. In the Clark Fork River, juvenile brown trout were most abundant in Reach 6 and in Reach 2 near the confluence of Rock Creek. We found few juvenile brown trout in Reaches 1, 3, 4, and 5.

We found significantly more adult brown trout in reference states than in test states (Figure 14). With flow and habitat differences removed from analyses, adult brown trout were 1.9 times more numerous in reference states than in test states. Biomass of trout was also 1.9 times greater in the references states than in test states when we removed flow and habitat differences (Figure 15). Adult brown trout numbers decreased in a downstream direction in the Clark Fork River (Figure 14). There was, however, a slight increase in adult numbers near the confluence of Rock Creek.

Rainbow Trout.

Rainbow trout were significantly more abundant in the reference states than in the test states (Figure 16). We found the greatest density of rainbow trout in Reaches 1 and 2, and a few in Reaches 4, 5, and 6. Reference states, which were matched with states in the Clark Fork River had 5.6 times more fish than test states, and 5.3 times more fish after we removed flow and habitat differences. Biomass of rainbow trout was 3.9 times greater in reference states than in test states (Figure 17). After we removed habitat and flow differences, biomass was 3.1 times greater in reference states than in test states.

Reference states had significantly more juvenile rainbow trout than did test states (Figure 18). We found juvenile rainbows only in Reaches 1, 2, and 6 in the Clark Fork

Figure 12.--Mean densities of juvenile brown trout in test and reference states.

Figure 13.--Mean biomasses of juvenile brown trout in test and reference states.

Adult Brown Trout

Fish/Hectare

Sign Test:
Prob T>R=0.001

Fish/WUA x 1000
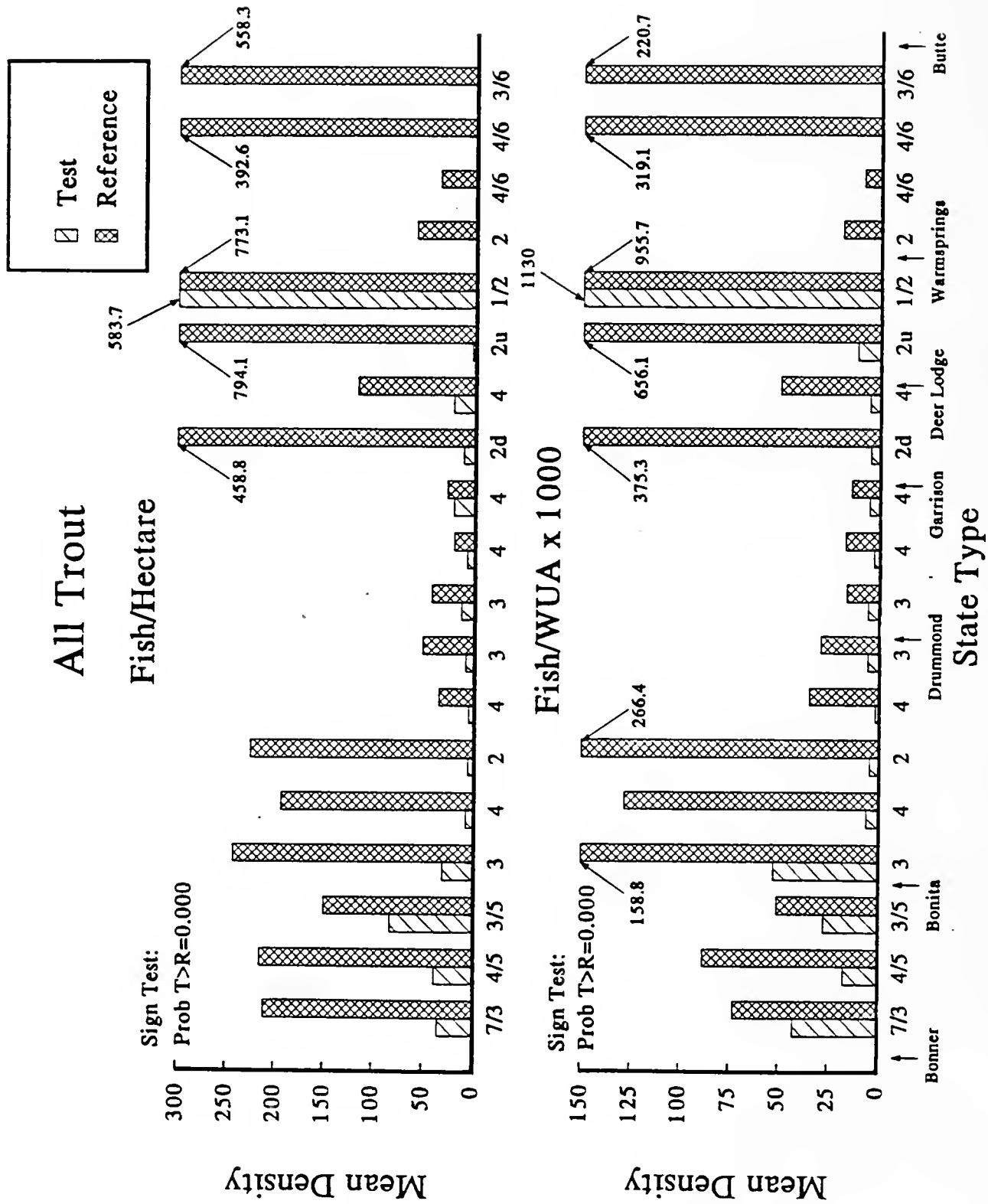
Sign Test:
Prob T>R=0.000
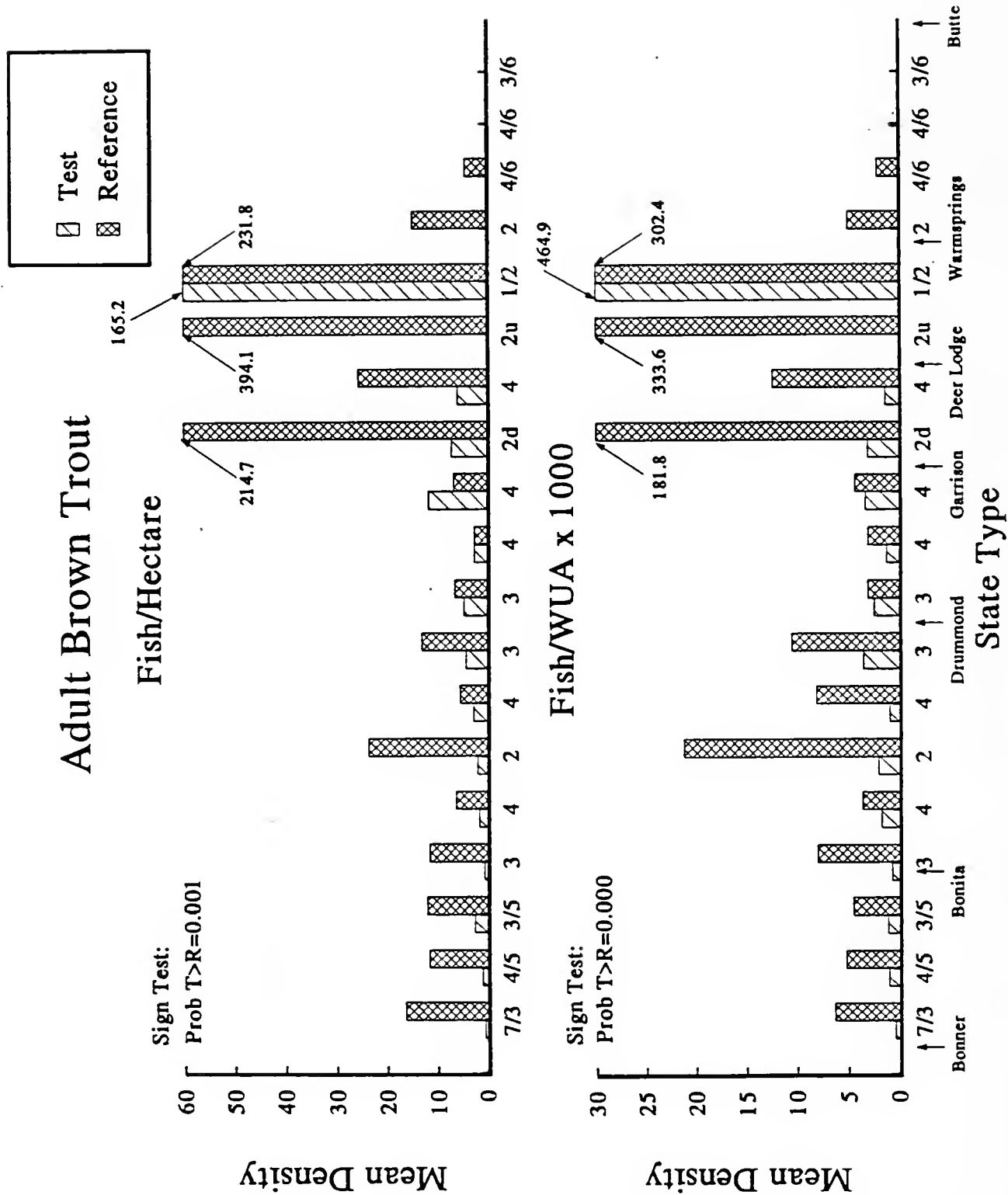
Figure 14.--Mean densities of adult brown trout in test and reference states.
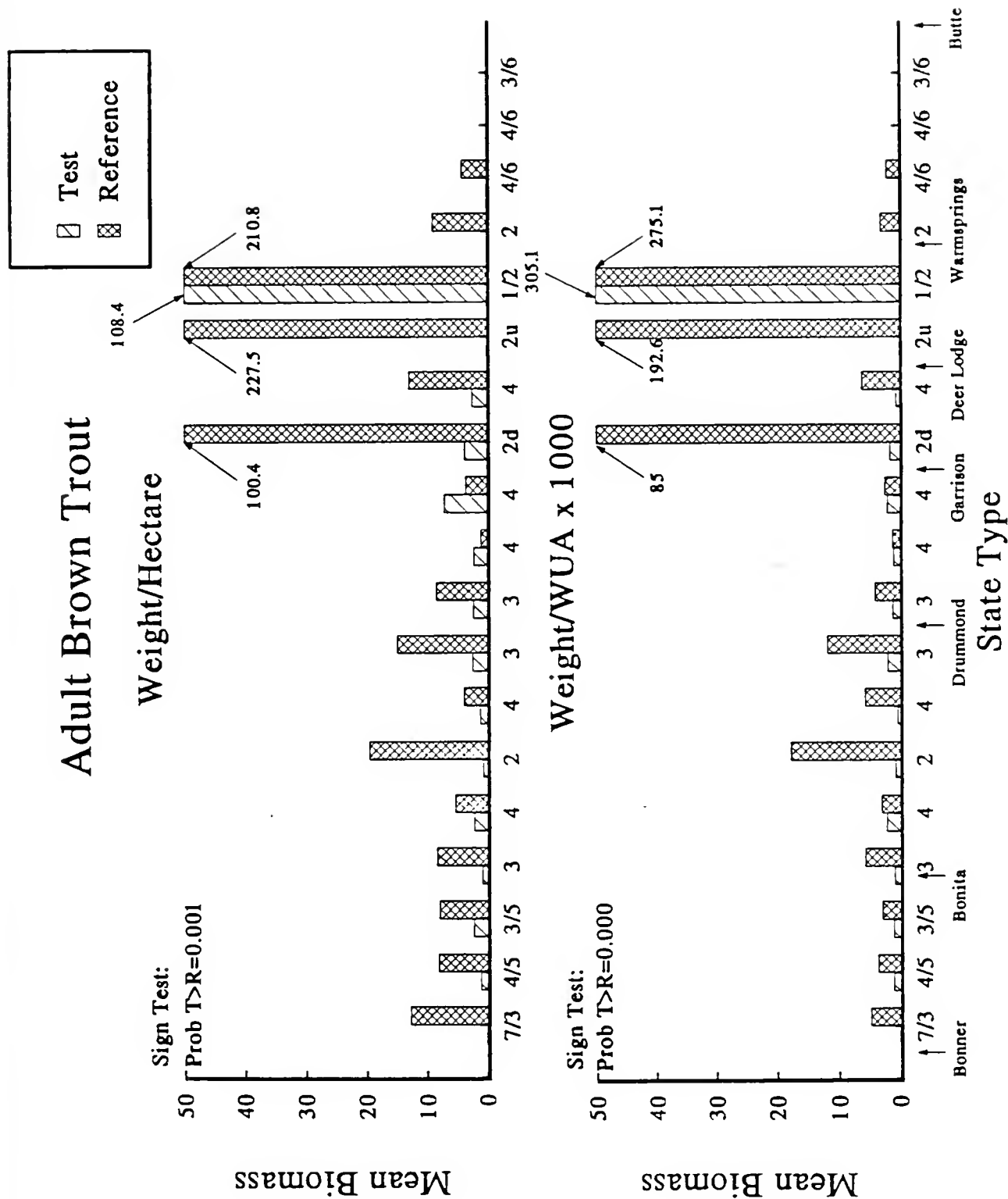
Figure 15.--Mean biomasses of adult brown trout in test and reference states.
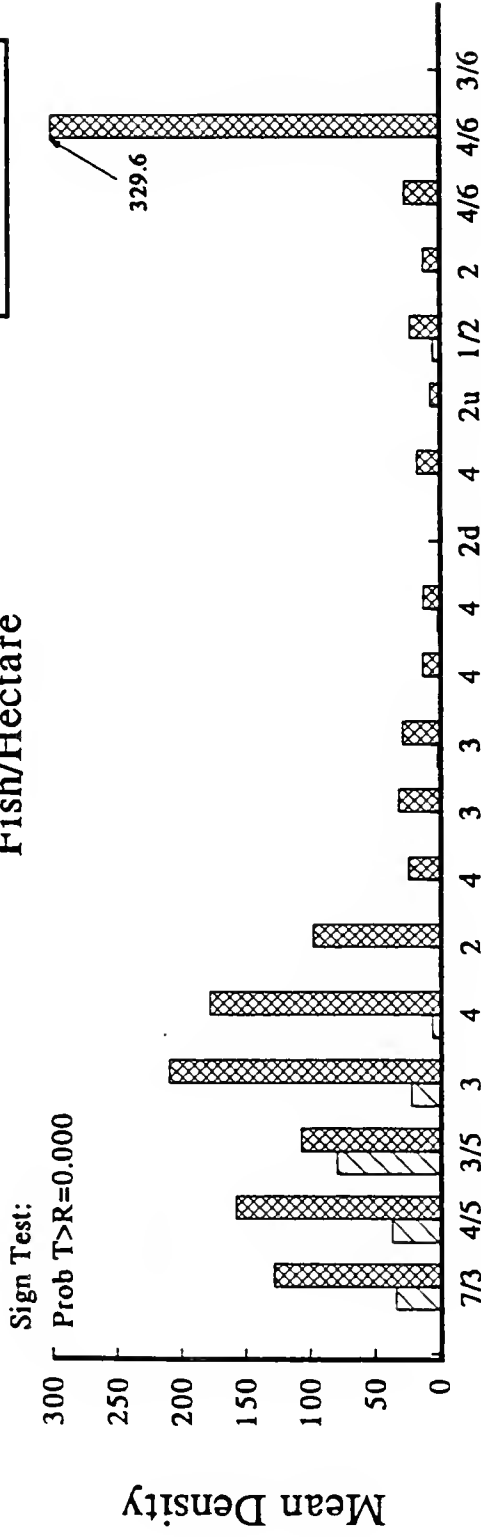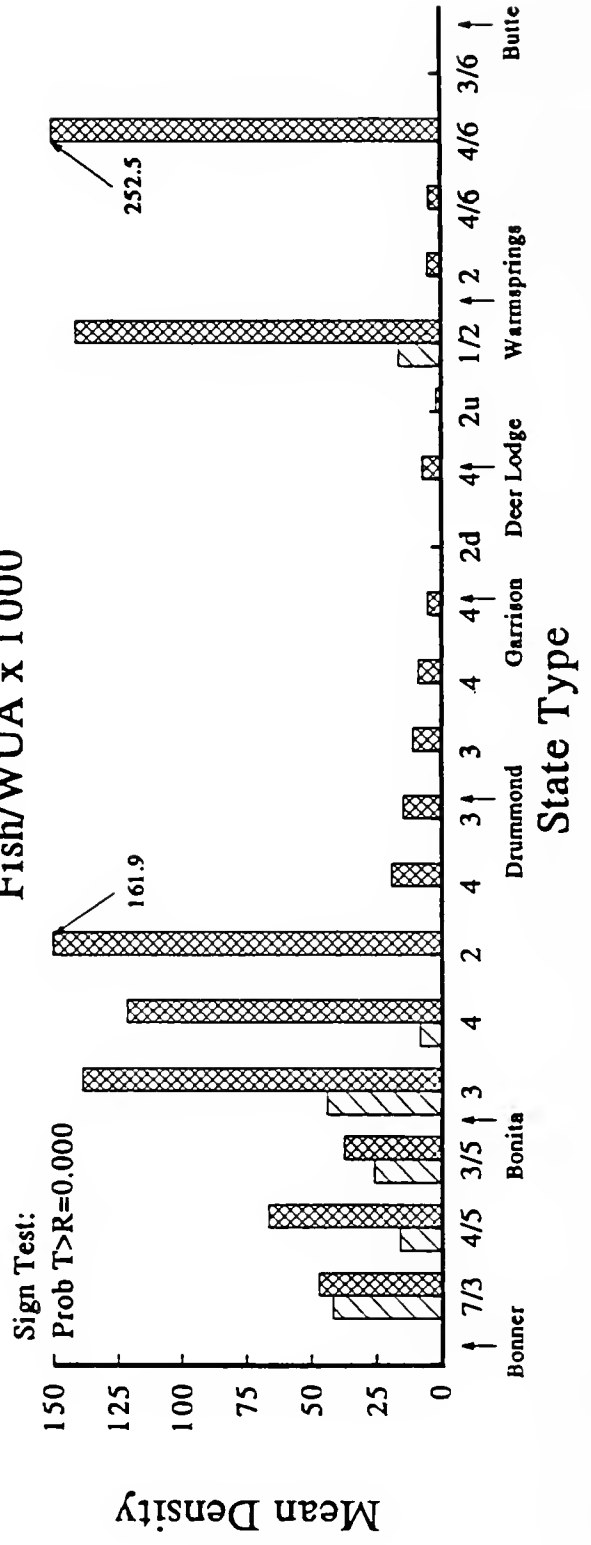
Figure 16.--Mean densities of all rainbow trout in test and reference states.
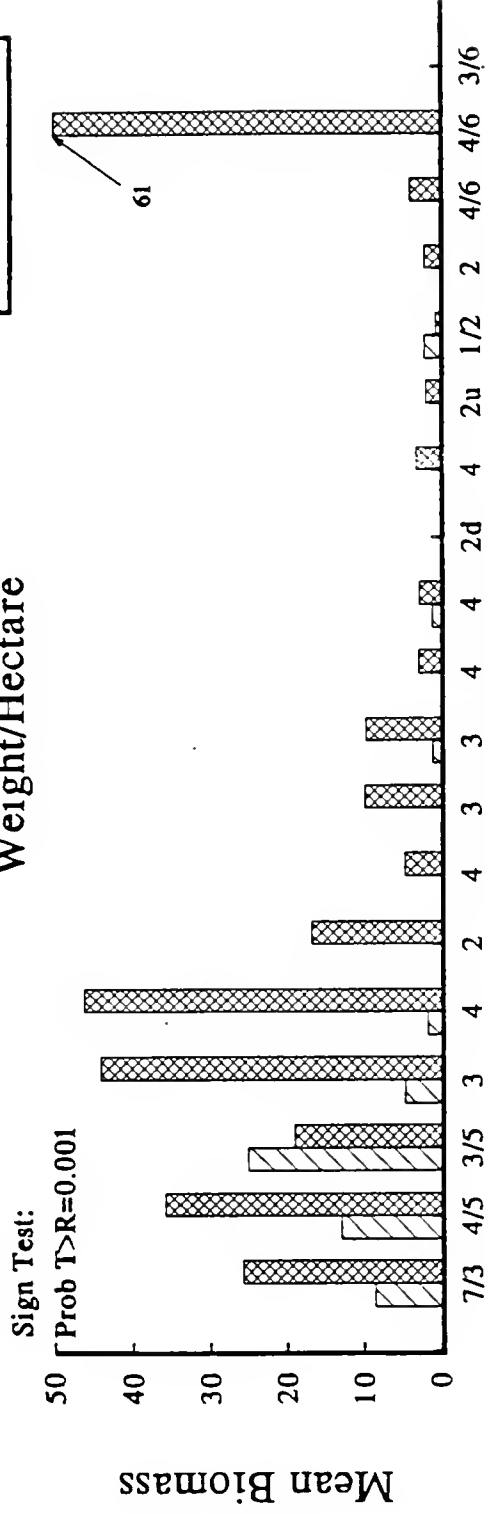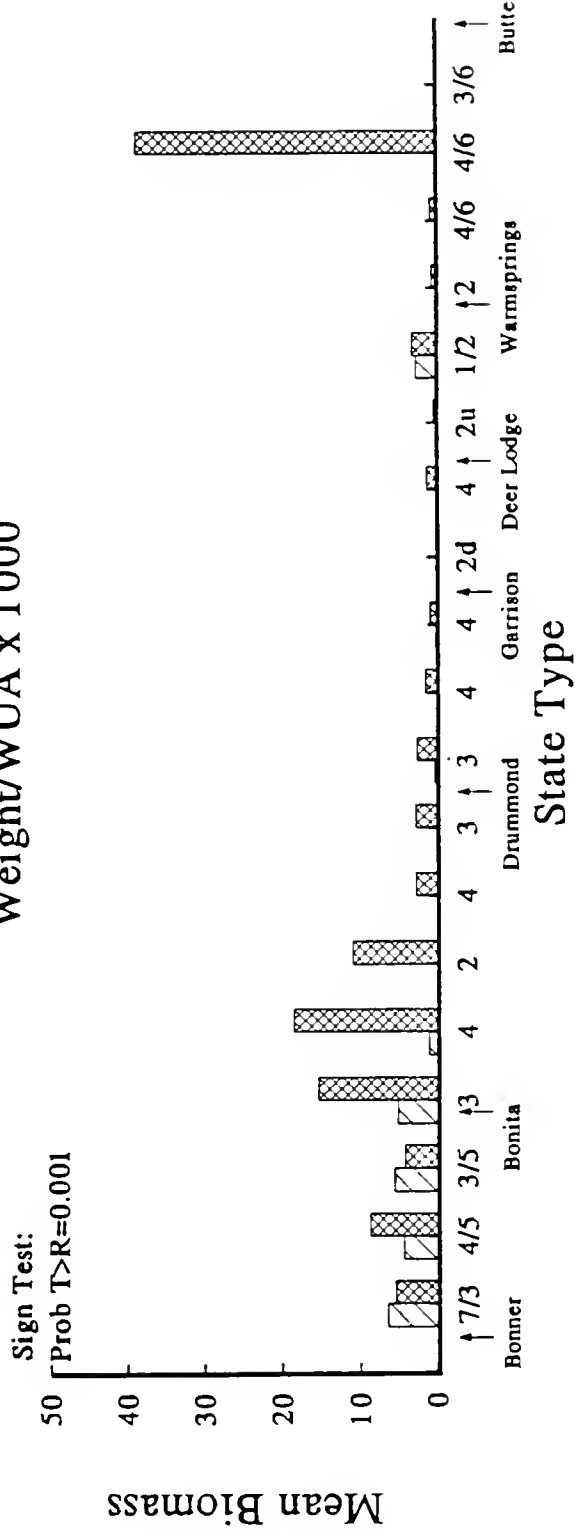
Figure 17.--Mean biomasses of all rainbow trout in test and reference states.
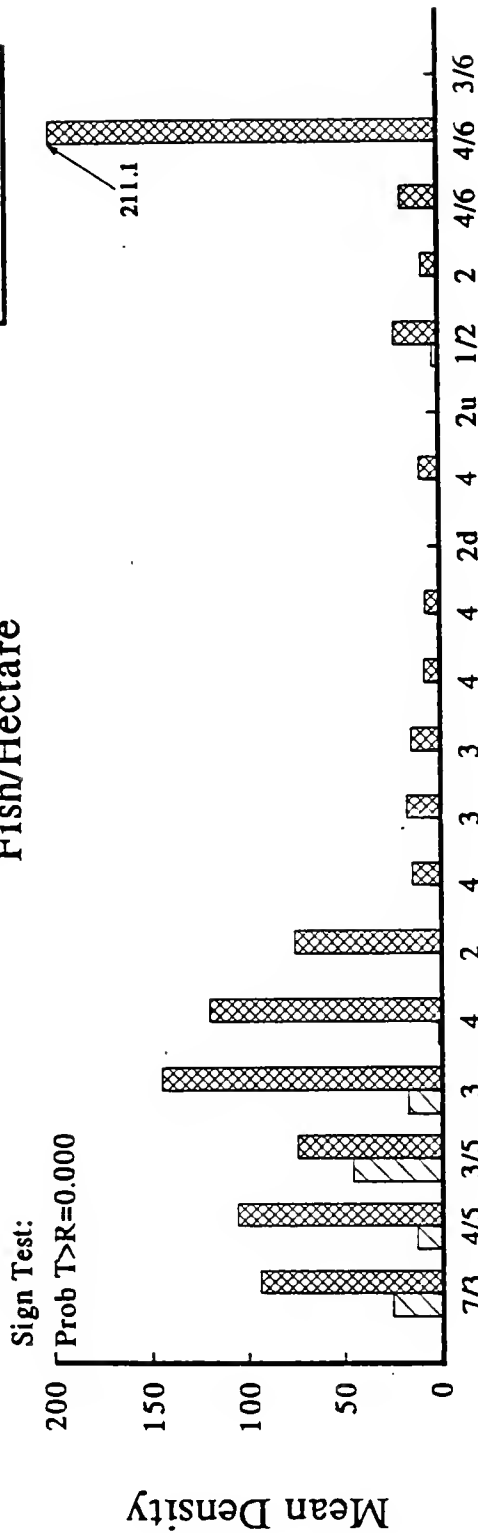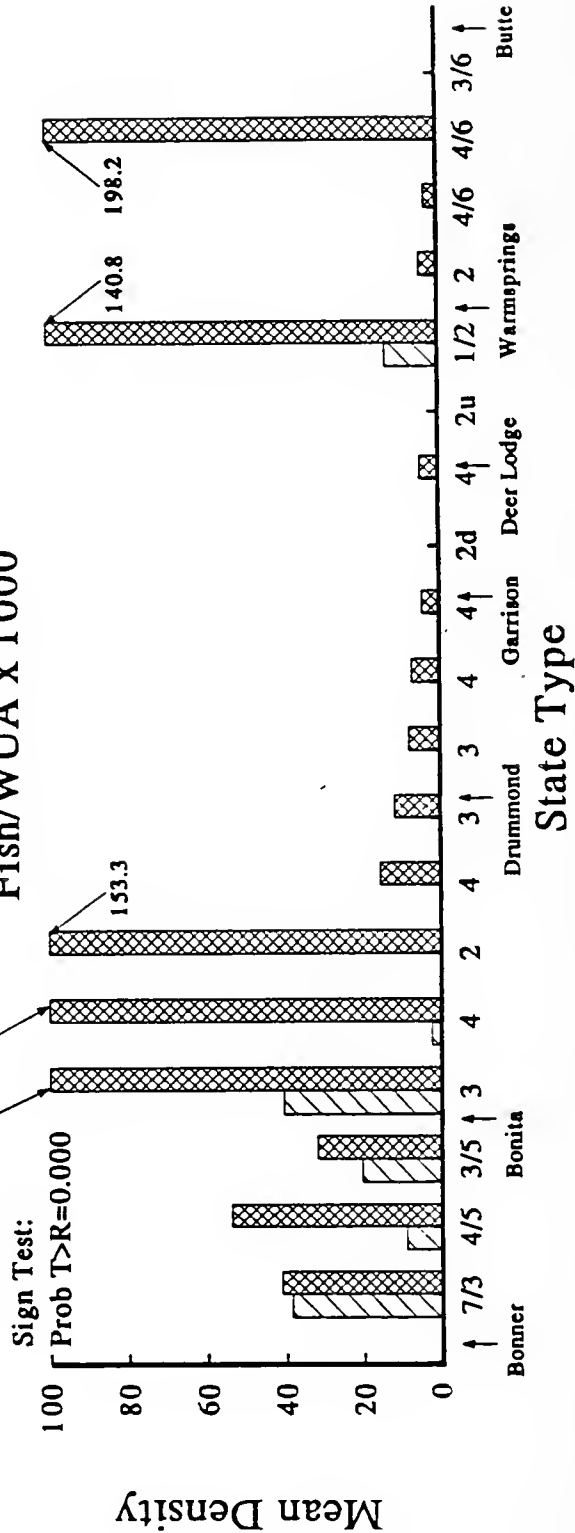
Figure 18.--Mean densities of juvenile rainbow trout in test and reference states.

River. Reference states had 5.6 times more juvenile rainbows than did test states after we removed habitat and flow differences. Biomass of juvenile rainbows was 3.1 times greater in reference states than in test states after habitat and flow differences were removed (Figure 19). Juvenile rainbows constituted 56% of all rainbow that we observed in the test states and 69% of the rainbows in reference states.

As with juvenile rainbow trout, reference states had significantly more adult rainbow trout than did test states (Figure 20). In the Clark Fork River, we found adult rainbows in Reaches 1 and 2 primarily, and a few in Reaches 4, 5, and 6. After we removed flow and habitat differences, adult rainbows were on average 3.2 times more abundant in reference states than in test states in the Clark Fork River (Figure 20). Biomass of adults in reference states was 3.0 times greater than in test states when habitat and flow differences were removed (Figure 21).

Brook Trout.

We found brook trout only in reference states that were matched with state types in Silver Bow Creek (Appendix F10). After we removed habitat and flow differences, brook trout numbered 66.6 to 220.7 fish/WUA in reference states. Biomass ranged from 6.8 to 11.0 pounds/WUA. Juvenile brook trout made up 95% of the total brook trout population, and their densities and biomasses ranged from 62.0 to 211.9 fish/WUA and 5.4 to 9.1 pounds/WUA, respectively. Densities and biomasses of adult brook trout ranged from 4.6 to 8.8 fish/WUA and 1.4 to 1.9 pounds/WUA, respectively.
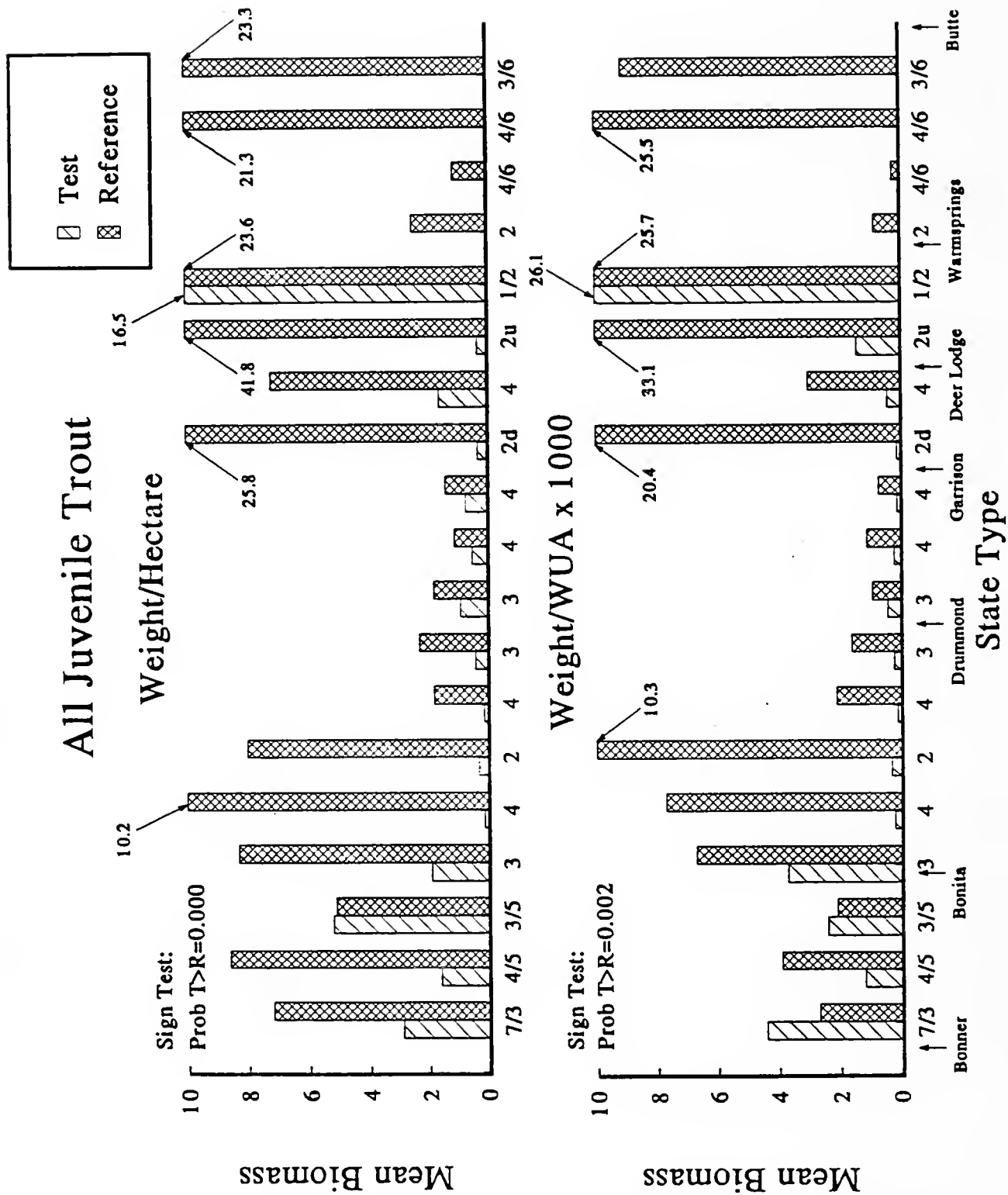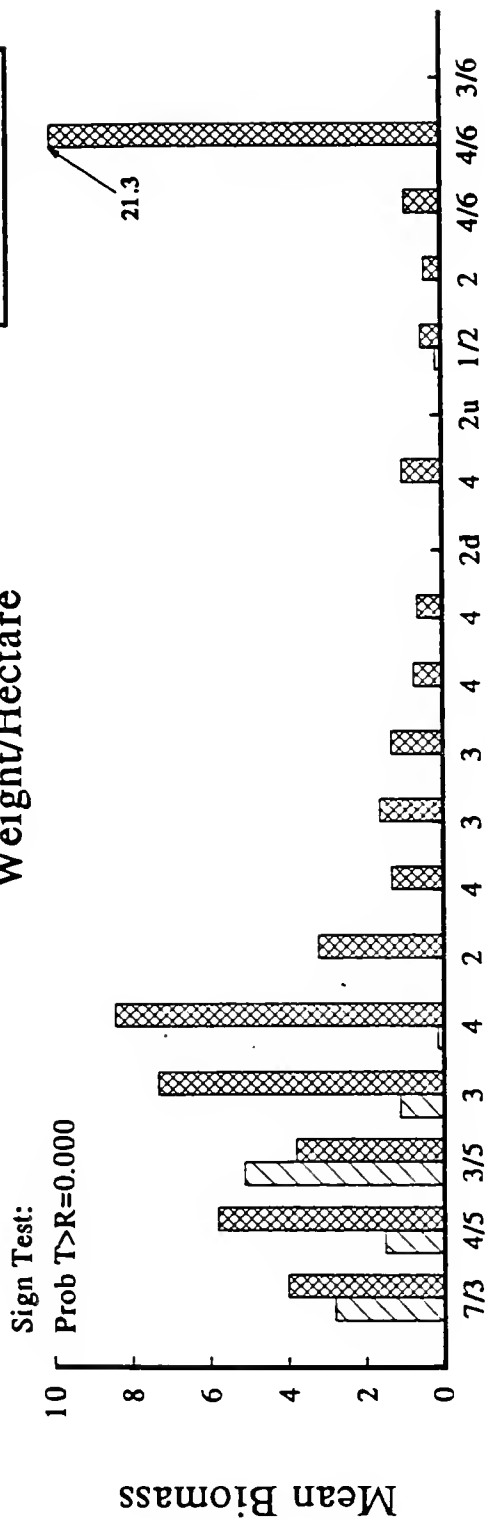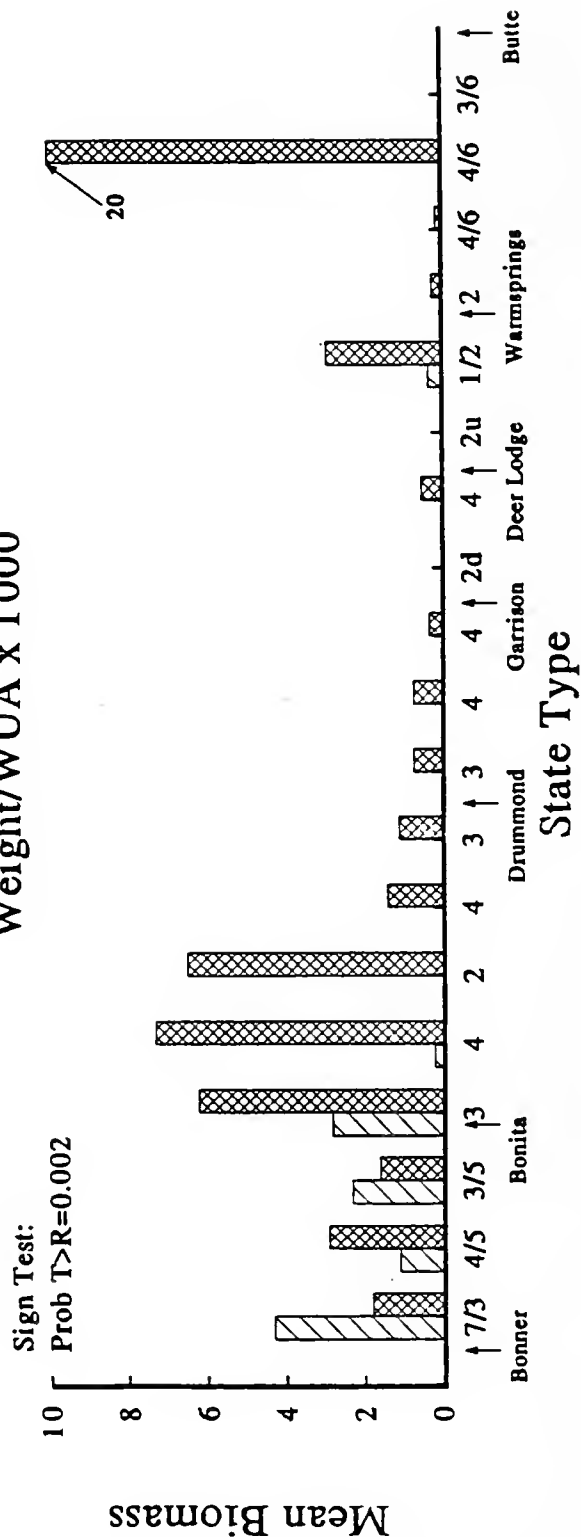
Figure 19.--Mean biomasses of juvenile rainbow trout in test and reference states.
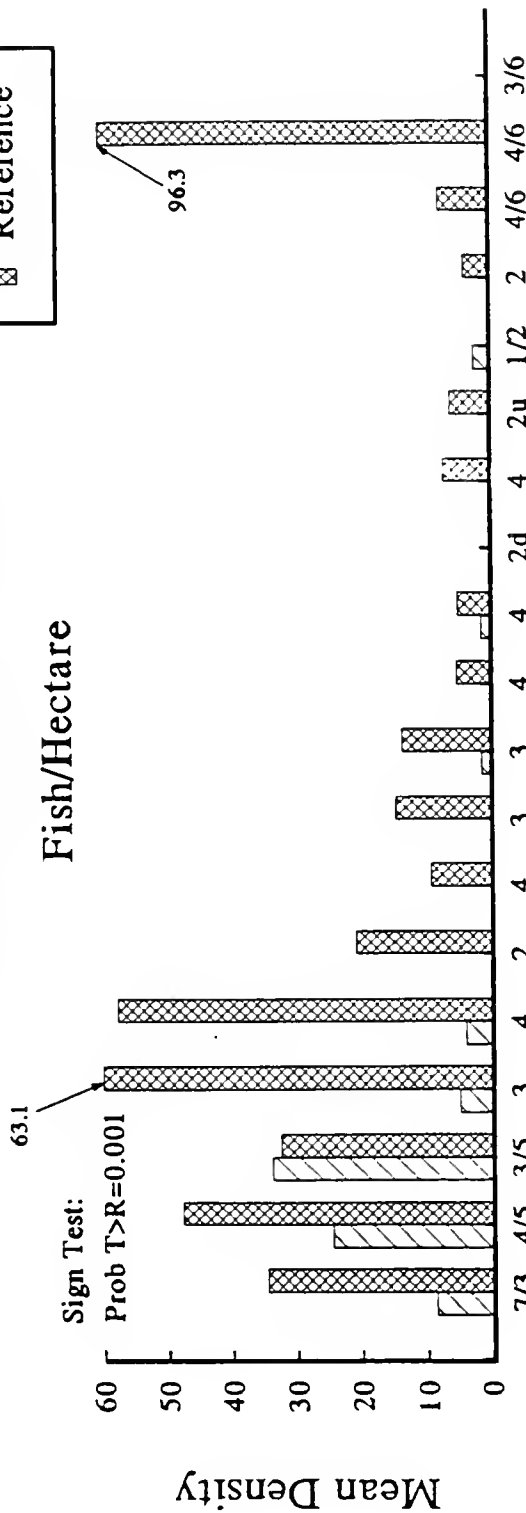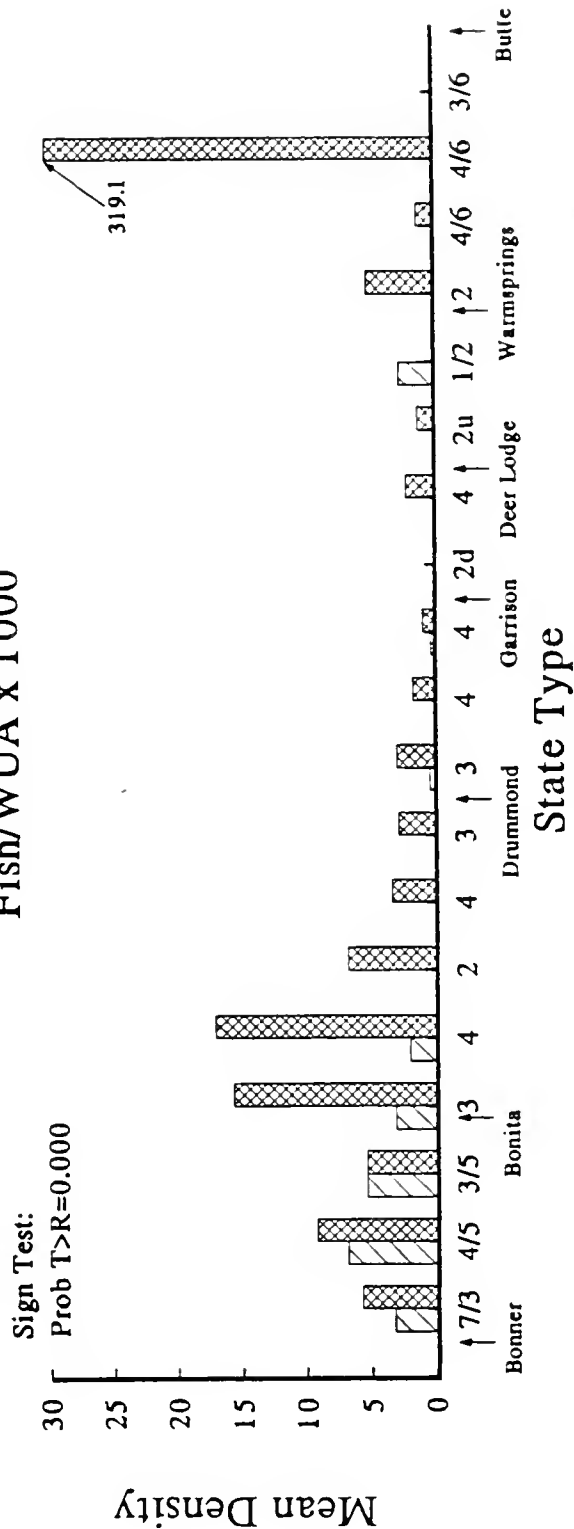
Figure 20.--Mean densities of adult rainbow trout in test and reference states.
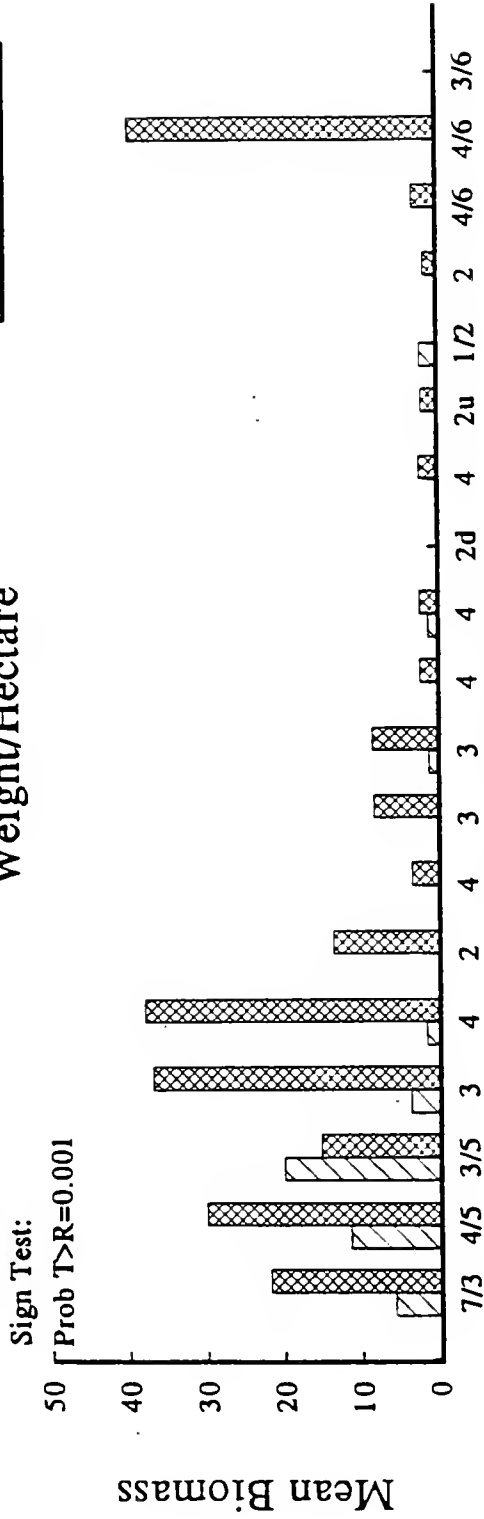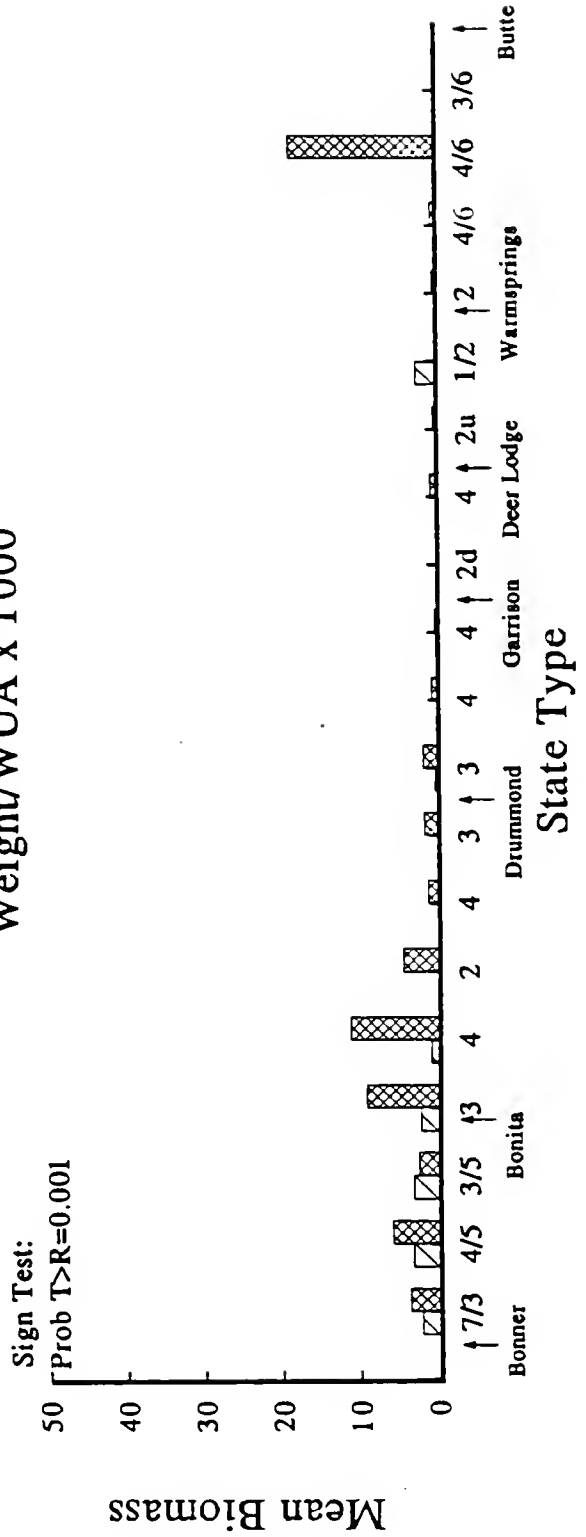
Figure 21.--Mean biomasses of adult rainbow trout in test and reference states.

DISCUSSION

We compared trout numbers and biomasses in the upper Clark Fork River and Silver Bow Creek with reference sites substantially unaffected by hazardous substances. Test and reference site pairings, based on stream classification, removed most of the variability associated with geology, land type, valley bottom type, and land and water uses. We compared paired sites classed as alike in our stratification scheme. Thus, for example, we paired a test site with laid-back banks (livestock damage) with a reference site in similar state after we otherwise classed them as similar with respect to geology, land type, and valley bottom type. We sought similar elevations and discharges. We had no knowledge in advance of fish populations present in test and reference sites.

We tested the hypothesis that fish abundance in test sites did not differ from that in reference sites. We rejected that hypothesis. Numbers and biomasses of trout in reference sites consistently and significantly exceeded those in the Clark Fork River and Silver Bow Creek. That difference held true for both juvenile and adult brown and rainbow trout. No trout lived in Silver Bow Creek, while reference sites supported rainbow, brown, or brook trout. We conclude that the differences between reference and Clark Fork and Silver Bow sites demonstrate real and statistically-significant effects of hazardous substances on trout populations in the former.

The magnitude of the difference between trout densities in Clark Fork and reference sites averaged 4.1-fold. Reference sites contained 3.9 times more juvenile trout than did test sites. Reference sites held 4.5-fold more adult trout (>8 inches) than did test sites. The great difference between test and control sites strongly indicates real effects of hazardous substances on Clark Fork trout populations.

We found significant differences between some components of habitat in some test and reference sites. Such differences will occur in nature in spite of the most

detailed classification and stratification. Shirvell and Dungey (1983) showed that brown trout preferred the same velocity for the same activity in all of six diverse rivers in New Zealand. Brown trout chose positions with optimum combinations of depth and velocity instead of positions with preferred values of either factor alone. We used physical habitat simulation (PHABSIM) modeling within the Instream Flow Incremental Model (IFIM) to adjust for habitat differences between test and reference sites. This model mates predicted depths, velocities and substrate in stream cells in test and reference sites with information on fish use of physical conditions (Bovee 1982). We adjusted for habitat conditions because even extensive classification and stratification cannot pair exact likes in test and reference sites. Flow and microhabitat differences remain after stratification. Flows, for example may not equate exactly in the test and reference sites. The PHABSIM model permits us to adjust for such differences. We used August streamflow for the final adjustment for discharge in each site, so that the model output consisted of weighted usable habitat at the lowest summer flow that we measured in summer of 1991.

We believe that the PHABSIM model accurately predicts locations that provide habitat within the stream. On the basis of extensive microhabitat measurements, Helm (1982) supports the view that one can predict locations used by brown trout from physical conditions. Gowan (1982) compared locations that brown trout used with PHABSIM predictions of usable habitat, and found strong correlations. Some workers (e.g., Orth and Maughan 1982; Mathur et al. 1985), however, have criticized PHABSIM because of unproven capability of the model to predict fish abundance. Much of the criticism of the model derives from failure to account for time series. Bovee (1988) demonstrated that instantaneous trout abundance results from events removed in time. However, we used PHABSIM output to standardize population estimates, not to predict fish abundance.

After adjusting for habitat differences and flows, we found 2.1 times more densities and biomass of all trout in reference sites than in contaminated sites in the Clark Fork. After we adjusted for habitat and flow differences, we found that reference sites held 1.9 times more adult trout and 2.0 times more trout biomass than did test sites. When we adjusted for habitat and flow differences, juvenile abundance averaged 2.4-fold greater in reference sites. When we compare fish densities and biomasses before habitat adjustments with numbers produced by the adjustment, we find that adjustment decreased the difference between test and reference sites. We cannot state, on the basis of our work, which hazardous substances singly or synergistically depress trout populations in the Clark Fork. The complete absence of fish in Silver Bow Creek provides especially distressing evidence that metals have eliminated fish from 29 miles of trout stream habitat.

On the basis of habitat availability and specific conductance (McFadden and Cooper 1962), we believe the Clark Fork should contain a higher standing crop of trout than reference sites. Conductance of Clark Fork water averages about three-fold greater than conductance of water in reference sites in five of seven Clark Fork reaches. Only in Reach 6 (Deerlodge, mean conductance = 546 micromhos) did the conductance not exceed that in the reference reach (Ruby River, mean conductance = 638 micromhos) (Appendix G).

We expect that the relatively low standing crops of adult brown trout in the Clark Fork sites, averaging 21.6% of the adult numbers in reference sites, reduce sport fishing catch rates, apart from effects on angler visits. Catch rates and total sustainable harvest should rise more or less linearly on average with standing stock of fish (Ricker 1975; Hilborn and Walters 1992). We suspect that angler visits would also correlate positively with stock density and catch rates (McFadden 1961). Thus, low standing stocks of trout in the Clark Fork reduce angler interest and use.

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APPENDIX A

Comparison of climatic, geologic, geomorphic, and hydrologic characters in test and reference reaches.

Appendix A1.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 1 in the Clark Fork River and its corresponding reference stream.

Character	Reach 1 Clark Fork	Reach 1 Rock Creek
Ecoregion	Northern Rockies	Northern Rockies
Geologic type	Resistant sedimentary	Resistant sedimentary
Landtype association	Fluvial lands	Fluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Meta-sedimentary canyon	Meta-sedimentary canyon
State types[1]	3/5; 4/5; 7/3; 8	2; 3; 10
Drainage area (sq miles)	3,641	885
Lower and upper elevations (ft)	3,255-3,499	3,499-3,980
Bottom width (ft)	3,000-4,000	2,000
Valley grade (%)	0.3	0.7
Stream sinuosity	1.12	1.10
Stream grade (%)	0.28	0.65
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C2	C2
Vegetation type	Cottonwoods	Cottonwoods
Mean discharge (cfs)	1,071	556
Peak discharge (cfs)	7,940	5,330
Base discharge (cfs)	233	48

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A2.--Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 2 in the Clark Fork River and its corresponding reference stream.

Character	Reach 2 Clark Fork	Reach 1 Rock Creek
Ecoregion	Northern Rockies	Northern Rockies
Geologic type	Resistant sedimentary	Resistant sedimentary
Landtype association	Fluvial lands	Fluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Meta-sedimentary canyon	Meta-sedimentary canyon
State types[1]	2/3; 3; 4	2; 3; 10
Drainage area (sq miles)	2,629	885
Lower and upper elevations (ft)	3,499-3,951	3,499-3,980
Bottom width (ft)	1,000-3,000	2,000
Valley grade (%)	0.2	0.7
Stream sinuosity	1.05	1.10
Stream grade (%)	0.23	0.65
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C2	C2
Vegetation type	Cottonwood	Cottonwoods
Mean discharge (cfs)	864	556
Peak discharge (cfs)	12,400	5,330
Base discharge (cfs)	59	48

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A3.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 3 in the Clark Fork River and its corresponding reference stream.

Character	Reach 3 Clark Fork	Reach 5 Big Hole
Ecoregion	Mont valley & foothill prairie	Mont valley & foothill prairie
Geologic type	Soft sedimentary	Soft sedimentary
Landtype association	Alluvial lands	Alluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Sedimentary valley	Sedimentary valley
State types[1]	3; 4	3; 5; 9/3; 10
Drainage area (sq miles)	2,378	2,476
Lower and upper elevations (ft)	3,890-3,954	4,850-5,060
Bottom width (ft)	3,000	6,000
Valley grade (%)	0.30	0.32
Stream sinuosity	1.17	1.17
Stream grade (%)	0.26	0.28
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C2	C2
Vegetation type	Cottonwood	Cottonwood
Mean discharge (cfs)	889	1,153
Peak discharge (cfs)	12,500	13,800
Base discharge (cfs)	58	6

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A4.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 4 in the Clark Fork River and its corresponding reference stream.

Character	Reach 4 Clark Fork	Reach 5 Big Hole
Ecoregion	Mont valley & foothill prairie	Mont valley & foothill prairie
Geologic type	Soft sedimentary	Soft sedimentary
Landtype association	Alluvial lands	Alluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Sedimentary valley	Sedimentary valley
State types[1]	3; 4	3; 5; 9/3; 10
Drainage area (sq miles)	1,704	2,762
Lower and upper elevations (ft)	3,890-3,954	4,850-5,060
Bottom width (ft)	3,000	6,000
Valley grade (%)	0.30	0.32
Stream sinuosity	1.17	1.17
Stream grade (%)	0.26	0.28
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C2	C2
Vegetation type	Cottonwood	Cottonwood
Mean discharge (cfs)	594	1,161
Peak discharge (cfs)	9,100	8,470
Base discharge (cfs)	55	55

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A5.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 5 in the Clark Fork River and its corresponding reference stream.

Character	Reach 5 Clark Fork	Reach 4 Big Hole
Ecoregion	Mont valley & foothill prairie	Mont valley & foothill prairie
Geologic type	Soft sedimentary	Soft sedimentary
Landtype association	Alluvial lands	Alluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Sedimentary canyon	Sedimentary canyon
State types[1]	4	11
Drainage area (sq miles)	1,704	2,476
Lower and upper elevations (ft)	4,200-4,336	4,822-4,850
Bottom width (ft)	1,000-2,000	3,000-4,000
Valley grade (%)	0.31	0.23
Stream sinuosity	1.06	1.08
Stream grade (%)	0.29	0.22
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C5	C5
Vegetation type	Cottonwood	Cottonwood
Mean discharge (cfs)	594	1,153
Peak discharge (cfs)	9,100	13,800
Base discharge (cfs)	55	6

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A6.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 6 in the Clark Fork River and its corresponding reference stream.

Character	Reach 6 Clark Fork	Reach 1 Flint Creek	Reach 1 Ruby
Ecoregion	Mont valley & fthill	Mont valley & fthill	Mont valley & fthill
Geologic type	Alluvium	Alluvium	Alluvium
Landtype association	Alluvial lands	Alluvial lands	Alluvial lands
Landtype	Valley-bottom	Valley-bottom	Valley-bottom
Valley-bottom type	Alluvial basin	Alluvial basin	Alluvial basin
State types[1]	1/2; 2; 3/4; 4; 11	2; 3	2; 4
Drainage area (sq miles)	1,139	490	935
Lower and upper elevations (ft)	4,336-4,790	3,954-4,295	4,636-5,240
Bottom width (ft)	2,000-3,000	1,000-2,000	4,000-8,000
Valley grade (%)	0.33	0.77	0.51
Stream sinuosity	1.63	1.29	1.96
Stream grade (%)	0.20	0.60	0.26
Dominant substrate	Rubble/gravel	Rubble/gravel	Rubble/gravel
Channel type[2]	C3	C3	C3
Vegetation type	Willow/hay	Willow/hay	Willow/hay
Mean discharge (cfs)	303	221	199
Peak discharge (cfs)	2,390	1,800	1,450
Base discharge (cfs)	22	10	2

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A6.--Concluded.

Character	Reach 6 Clark Fork	Reach 2 Beaverhead
Ecoregion	Mont valley & foothill prairie	Mont valley & foothill prairie
Geologic type	Alluvium	Alluvium
Landtype association	Alluvial lands	Alluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Alluvial basin	Alluvial basin
State types[1]	1/2; 2; 3/4; 4; 11	1/2
Drainage area (sq miles)	1,139	2,895
Lower and upper elevations (ft)	4,336-4,790	4,698-5,072
Bottom width (ft)	2,000-3,000	10,000-12,000
Valley grade (%)	0.33	0.32
Stream sinuosity	1.63	1.63
Stream grade (%)	0.20	0.20
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C3	C3
Vegetation type	Willow/hay	Willow/hay
Mean discharge (cfs)	303	396
Peak discharge (cfs)	2,390	1,700
Base discharge (cfs)	22	18

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A7.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 7 in Silver Bow Creek and its corresponding reference stream.

Character	Reach 7 Silver Bow	Reach 1 Big Hole	Reach 3 Ruby
Ecoregion	Mont valley & fthill	Mont valley & fthill	Mont valley & fthill
Geologic type	Alluvium	Alluvium	Alluvium
Landtype association	Alluvial lands	Alluvial lands	Alluvial lands
Landtype	Valley-bottom	Valley-bottom	Valley-bottom
Valley-bottom type	Alluvial basin	Alluvial basin	Alluvial basin
State types[1]	2; 4/6; 6	7; 3/9	2
Drainage area (sq miles)	483	2,762	596
Lower and upper elevations (ft)	4,790-5,106	4,605-4,770	5,400-5,840
Bottom width (ft)	5,000-6,000	16,000	4,000
Valley grade (%)	0.57	0.33	0.55
Stream sinuosity	1.15	1.22	1.68
Stream grade (%)	0.49	0.27	0.33
Dominant substrate	Rubble/gravel	Rubble/gravel	Rubble/gravel
Channel type[2]	C3	C3	C3
Vegetation type	Tailings	Cottonwood/hay	Willow/hay
Mean discharge (cfs)	148	1,161	220
Peak discharge (cfs)	1,220	8,470	2,500
Base discharge (cfs)	15	55	19

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A8.--Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 8 in Silver Bow Creek and its corresponding reference stream.

Character	Reach 8 Silver Bow	Reach 1 Bison Creek
Ecoregion	Mont valley & foothill prairie	Northern Rockies
Geologic type	Volcanic	Volcanic
Landtype association	Fluvial lands	Fluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Volcanic canyon	Volcanic canyon
State types[1]	4/6; 6	2/3
Drainage area (sq miles)	NA	NA
Lower and upper elevations (ft)	5,106-5,139	5,518-5,720
Bottom width (ft)	150-500	600
Valley grade (%)	0.62	0.86
Stream sinuosity	1.08	1.22
Stream grade (%)	0.58	0.70
Dominant substrate	Rubble/gravel	Rubble/gravel
Channel type[2]	C2	C2
Vegetation type	Tailings	Willow
Mean discharge (cfs)	NA	NA
Peak discharge (cfs)	NA	NA
Base discharge (cfs)	NA	NA

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A9.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 9 in Silver Bow Creek and its corresponding reference stream.

Character	Reach 9 Silver Bow	Reach 1 Bison Creek
Ecoregion	Mont valley & foothill prairie	Northern Rockies
Geologic type	Volcanic	Volcanic
Landtype association	Fluvial lands	Fluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Volcanic canyon	Volcanic canyon
State types[1]	4/6	2/3
Drainage area (sq miles)	NA	NA
Lower and upper elevations (ft)	5,139-5,254	5,518-5,720
Bottom width (ft)	150-500	600
Valley grade (%)	0.71	0.86
Stream sinuosity	1.07	1.22
Stream grade (%)	0.66	0.70
Dominant substrate	Sand	Rubble/gravel
Channel type[2]	C2	C2
Vegetation type	Tailings	Willow
Mean discharge (cfs)	NA	NA
Peak discharge (cfs)	NA	NA
Base discharge (cfs)	NA	NA

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

Appendix A10.—Comparison of climatic, geologic, geomorphic, and hydrologic characters between Reach 10 in Silver Bow Creek and its corresponding reference stream.

Character	Reach 10 Silver Bow	Reach 3 Bison Creek
Ecoregion	Mont valley & foothill prairie	Northern Rockies
Geologic type	Alluvial	Alluvial
Landtype association	Alluvial lands	Alluvial lands
Landtype	Valley-bottom landtype	Valley-bottom landtype
Valley-bottom type	Alluvial basin	Alluvial basin
State types[1]	3/6; 4; 4/6	2/3
Drainage area (sq miles)	103	NA
Lower and upper elevations (ft)	5,254-5,440	6,200-6,350
Bottom width (ft)	1,000-2,000	8,000
Valley grade (%)	0.34	0.29
Stream sinuosity	1.12	1.49
Stream grade (%)	0.31	0.19
Dominant substrate	Sand	Sand
Channel type[2]	C4	C6
Vegetation type	Tailings	Willow/hay
Mean discharge (cfs)	24	NA
Peak discharge (cfs)	100	NA
Base discharge (cfs)	13	NA

[1]See Table 2 for description of state codes.

[2]Described in Rosgen (1985).

APPENDIX B

Statistical differences of microhabitat variables between test and reference segments.

Appendix B1.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the braided segment with laid-back banks (state type 7/3) within Reach 1 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 0.15 with 16 and 43 degrees of freedom; P=0.00.

Variable	Test		Reference		99% CI on ratio		Sign
	Mean	1SD	Mean	1SD	Lower	Upper	
Channel width (ft)[1]	114.17	45.37	84.23	13.34	1.07	1.66	Yes
Wetted ch. width (ft)	92.30	35.25	69.47	13.88	1.05	1.63	Yes
Riffle width (ft)[1]	49.70	36.72	0.00	0.00	t=7.41;	P=0.00	Yes
Run width (ft)	36.13	50.11	64.60	6.96	0.17	0.95	Yes
Pool width (ft)	7.20	20.22	5.87	17.38	t=0.27;	P=0.78	No
Pool rating	1.03	1.97	0.93	1.91	t=0.20;	P=0.84	No
Boulder substrate (ft)	22.30	10.84	6.87	2.21	2.36	4.29	Yes
Rubble substrate (ft)[1]	61.47	31.46	60.07	12.05	0.75	1.31	No
Gravel substrate (ft)[1]	7.13	12.69	1.33	1.92	0.54	22.08	No
Fine substrate (ft)	0.00	0.00	0.9	1.73	t=2.85;	P=0.01	Yes
Mean depth (ft)	2.18	0.54	1.68	0.36	1.11	1.50	Yes
Thalweg depth (ft)	3.57	0.76	2.85	0.92	1.04	1.53	Yes
Canopy cover (%)	0.33	1.82	0.20	1.10	0.99	1.01	No
Woody debris (%)	0.00	0.00	0.73	1.86	0.99	1.01	No
Sun arc (deg)	124.17	9.54	114.43	16.71	1.00	1.18	No
Bank angle (deg)	159.83	5.67	155.45	10.30	0.99	1.07	No
Veg. overhang (in)	0.97	2.26	0.00	0.00	t=2.35;	P=0.03	No
Bank cover (%)	81.67	6.83	90.00	0.00	0.87	0.95	Yes
Bank alteration (%)	8.25	5.05	0.00	0.00	0.89	0.94	Yes
Bank undercut (in)	0.00	0.00	0.23	1.10	t=1.15;	P=0.26	No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

Appendix B2.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment with cut-off point bars (stream state 4/5) within Reach 1 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not by calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 30.39 with 11 and 48 degrees of freedom; $P = 0.00$.

	Test		Reference		99% CI on ratio		
Variable	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	96.30	31.64	84.23	13.34	0.94	1.36	No
Wetted ch. width (ft)	91.17	31.67	69.47	13.89	1.05	1.59	Yes
Riffle width (ft)[1]	25.33	54.87	0.00	0.00	t=2.53; P=0.02		No
Run width (ft)[1]	0.00	0.00	64.60	6.96	t=50.83; P=0.00		Yes
Pool width (ft)[1]	65.83	24.29	5.87	17.38	t=10.99; P=0.00		Yes
Pool rating	4.43	1.55	0.93	1.91	t=7.79; P=0.00		Yes
Boulder substrate (ft)	21.97	5.31	6.87	2.21	2.62	3.94	Yes
Rubble substrate (ft)[1]	69.20	27.99	60.07	12.05	0.90	1.43	No
Gravel substrate (ft)	0.00	0.00	1.33	1.92	t=3.81; P=0.00		Yes
Fine substrate (ft)	0.00	0.00	0.90	1.73	t=2.85; P=0.01		Yes
Mean depth (ft)	4.67	1.57	1.68	0.36	2.25	3.35	Yes
Thalweg depth (ft)[1]	7.88	2.11	2.86	0.92	2.23	3.43	Yes
Canopy cover (%)[1]	2.03	2.61	0.20	1.10	0.96	1.00	No
Woody debris (%)	0.00	0.00	0.73	1.86	0.99	1.02	No
Sun arc (deg)	141.27	1.51	114.43	16.71	1.15	1.33	Yes
Bank angle (deg)	146.05	11.19	155.45	10.30	0.89	0.99	Yes
Veg. overhang (in)	1.03	1.22	0.00	0.00	t=4.65; P=0.00		Yes
Bank cover (%)[2]	90.00	0.00	90.00	0.00	No CI		No
Bank alteration (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Bank undercut (in)	0.00	0.00	0.23	1.10	t=1.15; P=0.26		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B3.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the cut-off point bars segment with laid-back banks (stream state 3/5) within Reach 1 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 28.87 with 10 and 49 degrees of freedom; $P = 0.00$.

Variable	Test		Reference		99% CI on ratio		Sign
	Mean	1SD	Mean	1SD	Lower	Upper	
Channel width (ft)[1]	145.57	14.95	84.23	13.34	1.57	1.90	Yes
Wetted ch. width (ft)	132.23	19.49	69.47	13.89	1.68	2.16	Yes
Riffle width (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Run width (ft)[1]	0.00	0.00	64.60	6.96	$t=50.83$; $P=0.00$		Yes
Pool width (ft)[1]	132.23	19.49	5.87	17.38	$t=26.51$; $P=0.00$		Yes
Pool rating[1]	5.00	0.00	0.93	1.91	$t=11.66$; $P=0.00$		Yes
Boulder substrate (ft)	0.00	0.00	6.87	2.21	$t=17.03$; $P=0.00$		Yes
Rubble substrate (ft)[1]	127.77	14.17	60.07	12.05	1.90	2.39	Yes
Gravel substrate (ft)	0.73	1.39	1.33	1.92	0.03	2.28	No
Fine substrate (ft)	1.93	4.52	0.90	1.73	0.00	108.22	No
Mean depth (ft)[1]	3.28	0.22	1.68	0.36	1.76	2.17	Yes
Thalweg depth (ft)	5.17	0.71	2.86	0.92	1.52	2.18	Yes
Canopy cover (%)	0.56	1.50	0.20	1.10	0.98	1.01	No
Woody debris (%)	0.50	1.31	0.73	1.86	0.99	1.01	No
Sun arc (deg)[1]	140.17	5.36	114.43	16.71	1.13	1.32	Yes
Bank angle (deg)	165.42	4.11	155.45	10.30	1.03	1.10	Yes
Veg. overhang (in)	0.78	1.72	0.00	0.00	$t=2.46$; $P=0.02$		No
Bank cover (%)[2]	90.00	0.00	90.00	0.00	No CI		No
Bank alteration (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Bank undercut (in)	0.00	0.00	0.23	1.10	$t=1.15$; $P=0.26$		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B4.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the laid-back bank segment (stream state 3) within Reach 2 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 19.60 with 13 and 46 degrees of freedom; $P=0.00$.

	Test		Reference		99% CI on ratio		
Variable	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	128.63	49.70	98.10	7.22	1.05	1.57	Yes
Wetted ch. width (ft)	82.13	19.86	81.30	9.36	0.88	1.15	No
Riffle width (ft)	32.90	28.26	27.33	34.39	0.57	3.44	No
Run width (ft)	16.40	19.15	53.97	36.42	0.12	0.57	Yes
Pool width (ft)	34.17	32.02	0.00	0.00	t=5.84;	P=0.00	Yes
Pool rating[1]	3.27	1.17	0.00	0.00	t=15.26;	P=0.00	Yes
Boulder substrate (ft)	3.37	8.19	22.93	4.24	0.00	0.33	Yes
Rubble substrate (ft)	49.07	9.70	58.37	9.52	0.74	0.96	Yes
Gravel substrate (ft)	20.43	28.14	0.00	0.00	t=3.98;	P=0.00	Yes
Fine substrate (ft)	9.47	14.83	0.00	0.00	t=3.49;	P=0.00	Yes
Mean depth (ft)	1.49	0.69	1.35	0.31	0.81	1.42	No
Thalweg depth (ft)[1]	2.86	0.84	2.60	0.55	0.91	1.31	No
Canopy cover (%)	0.97	5.29	0.00	0.00	0.96	1.02	No
Woody debris (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Sun arc (deg)[1]	152.63	14.24	131.73	3.00	1.10	1.21	Yes
Bank angle (deg)	163.38	11.63	166.23	6.08	0.94	1.02	No
Veg. overhang (in)[2]	0.00	0.00	0.00	0.00	No CI		No
Bank cover (%)[2]	90.00	0.00	90.00	0.00	No CI		No
Bank alteration (%)	1.42	4.78	0.00	0.00	0.96	1.01	No
Bank undercut (in)	0.25	1.37	0.00	0.00	t=1.00;	P=0.33	No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B5.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment (stream state 4) within Reach 2 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace $= 21.75$ with 11 and 48 degrees of freedom; $P = 0.00$.

| Variable | Test | | Reference | | 99% CI on ratio | | |
	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	104.10	10.53	131.13	13.46	0.74	0.85	Yes
Wetted ch. width (ft)	74.97	14.17	119.80	16.54	0.55	0.70	Yes
Riffle width (ft)[1]	3.97	9.51	115.73	13.01	0.00	0.08	Yes
Run width (ft)[1]	66.80	12.54	2.67	7.46	t=24.07;	P=0.00	Yes
Pool width (ft)	4.23	9.70	2.07	5.95	t=1.04;	P=0.30	No
Pool rating	2.33	0.88	0.27	0.69	t=10.08;	P=0.00	No
Boulder substrate (ft)	0.60	1.59	14.63	9.21	0.00	0.10	Yes
Rubble substrate (ft)[1]	59.90	8.91	103.40	16.34	0.51	0.65	Yes
Gravel substrate (ft)	13.60	17.58	0.00	0.00	t=4.24;	P=0.00	Yes
Fine substrate (ft)	1.10	3.52	2.30	3.21	0.00	2.23	No
Mean depth (ft)	1.35	0.23	1.01	0.15	1.19	1.50	Yes
Thalweg depth (ft)	2.48	0.40	2.16	0.33	1.03	1.28	Yes
Canopy cover (%)[1]	0.00	0.00	2.03	4.83	0.99	1.04	No
Woody debris (%)	0.00	0.00	0.32	1.13	0.99	1.01	No
Sun arc (deg)[1]	150.07	5.85	110.30	12.63	1.28	1.44	Yes
Bank angle (deg)	166.98	8.29	151.92	12.61	1.05	1.15	Yes
Veg. overhang (in)	0.00	0.00	1.38	3.27	t=2.32;	P=0.03	No
Bank cover (%)[2]	90.00	0.00	90.00	0.00	No CI		No
Bank alteration (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Bank undercut (in)[2]	0.00	0.00	0.00	0.00	No CI		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B6.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the eroded banks segment (stream state 2) within Reach 2 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 6.45 with 13 and 46 degrees of freedom; P=0.00.

Variable	Test		Reference		99% CI on ratio		
	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)	101.07	10.30	111.20	12.89	0.84	0.98	Yes
Wetted ch. width (ft)	83.30	10.30	72.90	6.34	1.05	1.23	Yes
Riffle width (ft)[1]	0.00	0.00	22.90	22.13	t=5.67;	P=0.00	Yes
Run width (ft)[1]	83.30	10.30	38.03	25.26	1.62	3.30	Yes
Pool width (ft)	0.00	0.00	12.27	7.86	t=8.54;	P=0.00	Yes
Pool rating[1]	0.00	0.00	3.03	1.88	t=8.82;	P=0.00	Yes
Boulder substrate (ft)	7.67	2.90	15.47	6.87	0.37	0.67	Yes
Rubble substrate (ft)	60.77	13.77	56.63	8.74	0.93	1.22	No
Gravel substrate (ft)	12.80	8.08	0.60	1.10	9.96	263.45	Yes
Fine substrate (ft)	0.63	0.85	0.53	1.83	t=0.27;	P=0.79	No
Mean depth (ft)	1.33	0.32	1.72	0.55	0.63	0.95	Yes
Thalweg depth (ft)	2.83	0.81	3.03	0.72	0.77	1.12	No
Canopy cover (%)[1]	0.00	0.00	0.07	0.37	0.99	1.00	No
Woody debris (%)	0.00	0.00	1.60	5.23	0.99	1.04	No
Sun arc (deg)	142.47	5.05	124.17	11.05	1.09	1.21	Yes
Bank angle (deg)	166.03	5.56	166.50	7.65	0.96	1.03	No
Veg. overhang (in)	0.00	0.00	0.03	0.18	t=1.00;	P=0.33	No
Bank cover (%)[2]	90.00	0.00	90.00	0.00	No CI		No
Bank alteration (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Bank undercut (in)[2]	0.00	0.00	0.00	0.00	No CI		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B7.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment (stream state 4) within Reach 3 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 269.61 with 11 and 48 degrees of freedom; P=0.00.

Variable	Test		Reference		99% CI on ratio		Sign
	Mean	1SD	Mean	1SD	Lower	Upper	
Channel width (ft)[1]	136.13	7.93	200.90	9.23	0.65	0.70	Yes
Wetted ch. width (ft)	127.13	9.01	170.23	20.43	0.69	0.80	Yes
Riffle width (ft)[1]	0.90	1.92	142.43	55.28	0.00	0.01	Yes
Run width (ft)	126.23	9.09	0.00	0.00	t=7-.06; P=0.00		Yes
Pool width (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Pool rating[2]	0.00	0.00	0.00	0.00	No CI		No
Boulder substrate (ft)[1]	1.07	1.26	14.77	3.87	0.03	0.12	Yes
Rubble substrate (ft)[1]	98.30	24.77	155.77	18.35	0.54	0.72	Yes
Gravel substrate (ft)	14.27	18.11	0.00	0.00	t=4.32; P=0.00		Yes
Fine substrate (ft)[1]	11.00	11.06	0.00	0.00	t=5.45; P=0.00		Yes
Mean depth (ft)	0.87	0.12	0.74	0.12	1.06	1.29	Yes
Thalweg depth (ft)	1.66	0.23	1.73	0.26	0.86	1.07	No
Canopy cover (%)	0.40	1.54	0.43	1.30	0.99	1.01	No
Woody debris (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Sun arc (deg)	159.63	4.08	146.57	7.87	1.06	1.12	Yes
Bank angle (deg)	158.07	11.57	172.25	2.69	0.88	0.95	Yes
Veg. overhang (in)	0.12	0.55	0.35	1.16	t=0.99; P=0.32		No
Bank cover (%)[1]	86.55	10.68	90.00	0.00	0.90	1.02	No
Bank alteration (%)	4.25	13.03	0.00	0.00	0.89	1.02	No
Bank undercut (in)	0.45	2.46	0.00	0.00	t=1.00; P=0.33		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B8.--Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the laid-back banks segment (stream state 3) within Reach 3 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 12.44 with 13 and 46 degrees of freedom; $P = 0.00$.

	Test		Reference		99% CI on ratio		
Variable	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	106.27	11.83	160.07	6.97	0.62	0.70	Yes
Wetted ch. width (ft)	94.97	15.72	103.57	8.50	0.83	1.00	No
Riffle width (ft)	43.90	20.73	0.00	0.00	t=11.59;	P=0.00	Yes
Run width (ft)[1]	41.87	27.60	99.03	10.42	0.28	0.57	Yes
Pool width (ft)	9.40	15.87	4.60	11.92	t=1.32;	P=0.19	No
Pool rating[1]	1.53	2.22	0.60	1.56	t=1.88;	P=0.07	No
Boulder substrate (ft)	4.57	2.05	8.73	3.44	0.38	0.71	Yes
Rubble substrate (ft)	80.87	12.89	94.83	6.86	0.78	0.93	Yes
Gravel substrate (ft)	3.03	3.10	0.00	0.00	t=5.36;	P=0.00	Yes
Fine substrate (ft)	6.27	8.25	0.00	0.00	t=4.16;	P=0.00	Yes
Mean depth (ft)	1.14	0.24	1.07	0.09	t=1.57;	P=0.12	No
Thalweg depth (ft)	2.33	0.49	1.97	0.15	1.04	1.32	Yes
Canopy cover (%)[1]	5.10	6.38	0.00	0.00	0.92	0.98	Yes
Woody debris (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Sun arc (deg)	126.00	14.77	146.13	2.85	0.81	0.91	Yes
Bank angle (deg)[1]	110.98	22.28	171.43	2.68	0.58	0.72	Yes
Veg. overhang (in)	2.37	2.75	0.00	0.00	t=4.72;	P=0.00	Yes
Bank cover (%)[1]	82.20	10.71	90.00	0.00	0.85	0.97	Yes
Bank alteration (%)	9.67	9.16	0.00	0.00	0.86	0.95	Yes
Bank undercut (in)	0.58	0.49	0.00	0.00	t=6.46;	P=0.00	Yes

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B9.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the laid-back banks segment (stream state 3) within Reach 4 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 25.05 with 12 and 47 degrees of freedom; $P = 0.00$.

	Test		Reference		99% CI on ratio		
Variable	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	86.30	15.71	125.77	6.92	0.62	0.75	Yes
Wetted ch. width (ft)	63.17	10.74	109.03	9.54	0.52	0.63	Yes
Riffle width (ft)[1]	32.03	33.73	0.00	0.00	t=5.20;	P=0.00	Yes
Run width (ft)[1]	3.73	11.42	109.03	9.54	0.00	0.09	Yes
Pool width (ft)	26.87	22.81	0.00	0.00	t=6.45;	P=0.00	Yes
Pool rating[1]	2.33	1.74	0.00	0.00	t=7.31;	P=0.00	Yes
Boulder substrate (ft)	0.30	0.70	2.57	2.34	0.00	0.32	Yes
Rubble substrate (ft)[1]	53.40	12.77	103.33	7.11	0.45	0.58	Yes
Gravel substrate (ft)	9.43	6.27	1.30	1.57	3.86	19.45	Yes
Fine substrate (ft)	1.23	2.97	1.93	1.46	0.00	1.63	No
Mean depth (ft)	1.14	0.49	1.71	0.18	0.52	0.82	Yes
Thalweg depth (ft)	2.11	0.81	2.71	0.22	0.62	0.94	Yes
Canopy cover (%)[1]	0.50	2.01	0.47	1.54	0.98	1.01	No
Woody debris (%)	0.30	1.02	0.00	0.00	0.99	1.00	No
Sun arc (deg)	157.63	7.03	159.07	6.13	0.96	1.02	No
Bank angle (deg)	169.95	7.19	166.88	2.66	0.99	1.04	No
Veg. overhang (in)	0.15	0.60	0.30	0.98	t=0.71;	P=0.48	No
Bank cover (%)[2]	90.00	0.00	90.00	0.00	No CI		No
Bank alteration (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Bank undercut (in)	0.93	4.27	0.00	0.00	t=1.19;	P=0.24	No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B10.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment (stream state 4) within Reach 4 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 28.29 with 10 and 49 degrees of freedom; $P = 0.00$.

Variable	Test Mean	1SD	Reference Mean	1SD	99% CI on ratio Lower	Upper	Sign
Channel width (ft)[1]	102.60	4.63	200.90	9.23	0.49	0.53	Yes
Wetted ch. width (ft)	93.27	3.91	170.23	20.43	0.51	0.59	Yes
Riffle width (ft)[1]	0.00	0.00	142.43	55.28	t=14.11; P=0.00		Yes
Run width (ft)[1]	93.27	3.91	0.00	0.00	t=130.57; P=0.00		Yes
Pool width (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Pool rating[2]	0.00	0.00	0.00	0.00	No CI		No
Boulder substrate (ft)	11.00	3.52	14.77	3.87	0.60	0.91	Yes
Rubble substrate (ft)[1]	75.73	6.47	155.77	18.35	0.45	0.52	Yes
Gravel substrate (ft)	3.73	3.03	0.00	0.00	t=6.75; P=0.00		Yes
Fine substrate (ft)	2.50	1.43	0.00	0.00	t=9.56; P=0.00		Yes
Mean depth (ft)[1]	1.07	0.11	0.74	0.12	1.32	1.58	Yes
Thalweg depth (ft)	1.89	0.14	1.73	0.27	0.99	1.19	No
Canopy cover (%)[1]	0.00	0.00	0.43	1.30	1.00	1.01	No
Woody debris (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Sun arc (deg)	150.57	5.54	146.57	7.87	0.99	1.06	No
Bank angle (deg)	155.80	11.66	172.25	2.69	0.87	0.94	No
Veg. overhang (in)	0.00	0.00	0.35	1.16	t=1.65; P=0.11		No
Bank cover (%)	87.50	5.08	90.00	0.00	0.94	1.00	No
Bank alteration (%)	2.58	4.48	0.00	0.00	0.95	1.00	No
Bank undercut (in)[2]	0.00	0.00	0.00	0.00	No CI		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B11.--Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment (stream state 4) within Reach 5 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 125.33 with 12 and 47 degrees of freedom; P=0.00.

| | Test | | Reference | | 99% CI on ratio | | |
Variable	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	106.60	12.59	169.27	4.93	0.59	0.67	Yes
Wetted ch. width (ft)	95.43	20.93	150.40	6.34	0.56	0.71	Yes
Riffle width (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Run width (ft)[1]	92.17	22.12	150.40	6.34	0.54	0.69	Yes
Pool width (ft)	1.37	4.25	0.00	0.00	t=1.76; P=0.09		No
Pool rating[1]	0.30	0.92	0.00	0.00	t=1.79; P=0.08		No
Boulder substrate (ft)	7.73	3.59	12.07	3.76	0.47	0.84	Yes
Rubble substrate (ft)[1]	62.37	17.02	134.47	4.94	0.40	0.53	Yes
Gravel substrate (ft)	10.27	7.97	3.90	4.07	1.37	5.87	Yes
Fine substrate (ft)	14.67	11.89	0.47	0.73	14.41	134.61	Yes
Mean depth (ft)[1]	1.53	0.30	0.86	0.09	1.59	1.98	Yes
Thalweg depth (ft)[1]	3.94	1.17	1.45	0.13	2.30	3.13	Yes
Canopy cover (%)[1]	1.00	1.72	0.00	0.00	0.98	1.00	No
Woody debris (%)	0.10	0.40	0.00	0.00	0.99	1.00	No
Sun arc (deg)	154.77	4.98	131.87	13.88	1.11	1.24	Yes
Bank angle (deg)	134.45	17.45	174.72	2.99	0.72	0.82	Yes
Veg. overhang (in)	0.45	0.74	0.00	0.00	t=3.30; P=0.00		Yes
Bank cover (%)	88.33	4.32	90.00	0.00	0.96	1.01	No
Bank alteration (%)	2.90	4.19	0.00	0.00	0.95	0.99	Yes
Bank undercut (in)	0.03	0.11	0.00	0.00	t=1.22; P=0.23		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B12.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the downstream eroded banks segment (stream state 2d) within Reach 6 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 38.54 with 15 and 43 degrees of freedom; $P = 0.00$.

Variable	Test Mean	1SD	Reference Mean	1SD	99% CI on ratio Lower	Upper	Sign
Channel width (ft)[1]	83.83	7.72	38.37	6.96	1.98	2.42	Yes
Wetted ch. width (ft)	71.30	7.16	31.73	7.18	1.99	2.56	Yes
Riffle width (ft)	5.13	12.20	11.10	15.74	0.00	1.97	No
Run width (ft)[1]	66.17	10.23	17.27	12.64	2.76	6.09	Yes
Pool width (ft)	0.00	0.00	3.33	7.87	t=2.32;	P=0.03	No
Pool rating[1]	0.00	0.00	0.73	1.51	t=2.66;	P=0.01	Yes
Boulder substrate (ft)	0.00	0.00	7.90	6.67	t=6.49;	P=0.00	Yes
Rubble substrate (ft)	33.83	12.53	21.27	9.96	1.18	2.18	Yes
Gravel substrate (ft)[1]	17.03	4.90	0.67	1.18	13.05	267.71	Yes
Fine substrate (ft)	20.40	12.19	2.23	3.22	4.73	34.34	Yes
Mean depth (ft)	1.10	0.28	0.83	0.23	1.10	1.59	Yes
Thalweg depth (ft)	2.42	0.35	1.54	0.44	1.35	1.85	Yes
Canopy cover (%)	0.00	0.00	0.73	1.19	1.00	1.01	No
Woody debris (%)	0.00	0.00	0.16	0.47	0.99	1.00	No
Sun arc (deg)[1]	161.77	3.88	77.03	39.24	1.67	2.82	Yes
Bank angle (deg)	142.65	17.88	135.15	30.26	0.93	1.21	No
Veg. overhang (in)	0.00	0.00	3.17	4.77	t=3.58;	P=0.00	Yes
Bank cover (%)	79.08	12.08	89.58	2.28	0.81	0.95	Yes
Bank alteration (%)	8.58	9.25	11.22	15.20	0.93	1.14	No
Bank undercut (in)	0.07	0.13	2.00	3.43	0.01	0.28	Yes

[1]Variable not used in multivariate analysis because it was correlated with other variables.

Appendix B13.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment (stream state 4) within Reach 6 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 47.75 with 12 and 47 degrees of freedom; P=0.00.

Variable	Test		Reference		99% CI on ratio		
	Mean	1SD	Mean	1SD	Lower	Upper	Sign
Channel width (ft)[1]	84.77	7.95	40.30	7.07	1.91	2.33	Yes
Wetted ch. width (ft)	83.40	8.91	28.47	5.88	2.61	3.31	Yes
Riffle width (ft)	14.30	19.98	6.90	11.17	0.54	11.78	No
Run width (ft)[1]	69.10	15.51	11.90	13.18	3.66	13.21	Yes
Pool width (ft)	0.00	0.00	8.53	10.87	t=4.29;	P=0.00	Yes
Pool rating[1]	0.00	0.00	2.10	2.34	t=4.92;	P=0.00	Yes
Boulder substrate (ft)	0.00	0.00	0.07	0.25	t=1.44;	P=0.16	No
Rubble substrate (ft)	21.00	13.62	23.17	6.79	0.60	1.25	No
Gravel substrate (ft)[1]	46.73	9.67	2.73	3.17	10.64	41.46	Yes
Fine substrate (ft)	15.60	6.46	2.63	3.24	3.46	15.74	Yes
Mean depth (ft)[1]	0.59	0.07	0.90	0.53	0.51	0.91	Yes
Thalweg depth (ft)	1.34	0.30	1.46	0.79	0.71	1.26	No
Canopy cover (%)	4.40	5.08	9.43	18.73	0.95	1.18	No
Woody debris (%)	0.35	0.84	3.20	10.83	0.97	1.09	No
Sun arc (deg)	152.43	4.97	121.10	19.89	1.16	1.37	Yes
Bank angle (deg)[1]	68.17	32.10	146.05	31.24	0.35	0.59	Yes
Veg. overhang (in)	1.93	2.07	1.40	2.89	t=0.82;	P=0.42	No
Bank cover (%)[1]	8c 17	3.17	90.00	0.00	0.97	1.01	No
Bank alteration (%)	0.58	2.24	0.00	0.00	0.98	1.01	No
Bank undercut (in)[1]	0.87	0.35	0.03	0.14	t=12.17;	P=0.00	Yes

[1]Variable not used in multivariate analysis because it was correlated with other variables.

Appendix B14.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the upstream eroded banks segment (stream state 2u) within Reach 6 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 31.72 with 16 and 41 degrees of freedom; P=0.00.

Variable	Test Mean	1SD	Reference Mean	1SD	99% CI on ratio Lower	Upper	Sign
Channel width (ft)[1]	82.13	11.09	38.37	6.96	1.91	2.40	Yes
Wetted ch. width (ft)	75.43	10.11	31.73	7.18	2.09	2.72	Yes
Riffle width (ft)	20.17	24.44	11.10	15.74	0.62	6.76	No
Run width (ft)	48.73	20.52	17.27	12.64	1.90	4.63	Yes
Pool width (ft)	3.40	7.42	3.33	7.87	t=0.18; P=0.86		No
Pool rating[1]	0.90	1.86	0.73	1.51	t=0.38; P=0.71		No
Boulder substrate (ft)	0.00	0.00	7.90	6.67	t=6.49; P=0.00		Yes
Rubble substrate (ft)[1]	55.57	12.40	21.27	9.96	2.05	3.48	Yes
Gravel substrate (ft)	9.20	12.13	0.67	1.18	3.93	147.75	Yes
Fine substrate (ft)	7.63	4.71	2.23	3.22	1.76	12.85	Yes
Mean depth (ft)	0.75	0.24	0.83	0.23	0.74	1.10	No
Thalweg depth (ft)	1.78	0.51	1.54	0.44	0.95	1.41	No
Canopy cover (%)	1.40	3.05	0.73	1.19	0.97	1.01	No
Woody debris (%)	0.16	0.45	0.16	0.47	0.99	1.00	No
Sun arc (deg)[1]	164.27	7.00	77.03	39.24	1.69	2.86	Yes
Bank angle (deg)	112.92	40.75	135.15	30.26	0.66	1.03	No
Veg. overhang (in)	0.00	0.00	3.17	4.77	t=3.58; P=0.00		Yes
Bank cover (%)	70.37	18.08	89.58	2.28	0.68	0.89	Yes
Bank alteration (%)	25.33	22.56	11.22	15.20	0.70	0.99	Yes
Bank undercut (in)	0.24	0.37	2.00	3.43	0.02	0.95	Yes

[1]Variable not used in multivariate analysis because it was correlated with other variables.

Appendix B15.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the natural channel with eroded banks segment (stream state 1/2) within Reach 6 in the Clark Fork River and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 10.17 with 18 and 41 degrees of freedom; $P=0.00$.

Variable	Test Mean	Test 1SD	Reference Mean	Reference 1SD	99% CI on ratio Lower	99% CI on ratio Upper	Sign
Channel width (ft)	48.37	4.69	48.60	8.41	0.90	1.10	No
Wetted ch. width (ft)	32.57	8.81	48.60	8.41	0.57	0.78	Yes
Riffle width (ft)	8.60	11.47	0.00	0.00	t=4.11; P=0.00		Yes
Run width (ft)	9.65	11.39	30.73	12.51	0.13	0.53	Yes
Pool width (ft)	14.62	12.88	17.87	13.54	0.42	1.49	No
Pool rating	2.30	1.97	4.43	1.52	0.29	0.78	Yes
Boulder substrate (ft)	0.47	0.82	0.23	0.77	t=1.14; P=0.26		No
Rubble substrate (ft)	11.67	6.67	8.37	10.46	0.77	3.85	No
Gravel substrate (ft)	11.93	8.74	18.93	7.69	0.38	0.93	Yes
Fine substrate (ft)	9.13	9.37	21.07	12.18	0.20	0.74	Yes
Mean depth (ft)[1]	0.86	0.30	2.12	0.40	0.32	0.50	Yes
Thalweg depth (ft)	1.74	0.43	4.12	1.24	0.35	0.52	Yes
Canopy cover (%)	29.10	14.35	4.40	5.34	0.66	0.82	Yes
Woody debris (%)	4.33	6.19	2.67	4.11	0.95	1.02	No
Sun arc (deg)[1]	118.57	20.74	156.73	4.10	0.69	0.82	Yes
Bank angle (deg)	105.00	18.02	92.60	23.47	0.98	1.33	No
Veg. overhang (in)	0.48	0.27	2.14	2.69	0.12	0.62	Yes
Bank cover (%)	92.22	4.06	92.75	3.17	0.97	1.02	No
Bank alteration (%)	10.27	1.55	9.17	4.93	0.96	1.02	No
Bank undercut (in)	2.49	9.75	0.54	0.51	t=1.09; P=0.28		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

Appendix B16.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the eroded banks segment (stream state 2) within Reach 7 in Silver Bow Creek and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 20.15 with 13 and 46 degrees of freedom; P=0.00.

Variable	Test Mean	1SD	Reference Mean	1SD	99% CI on ratio Lower	Upper	Sign
Channel width (ft)[1]	33.50	4.45	52.87	3.48	0.59	0.68	Yes
Wetted ch. width (ft)	27.97	10.06	44.07	5.79	0.51	0.76	Yes
Riffle width (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Run width (ft)[1]	27.96	10.06	43.17	7.41	0.52	0.78	Yes
Pool width (ft)	0.00	0.00	0.90	3.45	t=1.43; P=0.16		No
Pool rating[1]	0.00	0.00	0.30	1.15	t=1.43; P=0.16		No
Boulder substrate (ft)	0.43	1.04	1.27	1.44	0.00	1.07	No
Rubble substrate (ft)	18.30	5.41	32.93	6.16	0.46	0.66	Yes
Gravel substrate (ft)	2.37	2.47	2.53	2.86	0.40	2.36	No
Fine substrate (ft)	5.13	4.64	7.53	5.61	0.34	1.24	No
Mean depth (ft)	1.05	0.24	1.31	0.19	0.69	0.92	Yes
Thalweg depth (ft)[1]	1.84	0.52	2.28	0.43	0.68	0.95	Yes
Canopy cover (%)[1]	7.76	8.03	3.17	8.33	0.89	1.01	No
Woody debris (%)	0.00	0.00	1.97	4.44	0.99	1.04	No
Sun arc (deg)	151.50	11.73	154.10	8.67	0.93	1.03	No
Bank angle (deg)	115.73	31.55	143.98	24.67	0.67	0.94	Yes
Veg. overhang (in)	0.97	0.99	0.63	1.57	t=0.98; P=0.33		No
Bank cover (%)[1]	83.30	11.91	71.85	8.43	1.05	1.27	Yes
Bank alteration (%)	6.40	12.54	24.42	12.12	1.12	1.38	Yes
Bank undercut (in)	0.19	0.27	0.09	0.23	t=1.61; P=0.11		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B17.--Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment with laid-back banks and mine tailings (stream state 4/6) within Reach 7 in Silver Bow Creek and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with P$\leq$0.01 as statistically significant. The multivariate Hotelling-Lawley trace = 148.23 with 12 and 47 degrees of freedom; P=0.00.

Variable	Test Mean	Test 1SD	Reference Mean	Reference 1SD	99% CI on ratio Lower	99% CI on ratio Upper	Sign
Channel width (ft)[1]	27.87	3.93	161.80	7.02	0.16	0.19	Yes
Wetted ch. width (ft)	26.37	3.79	79.43	7.47	0.30	0.36	Yes
Riffle width (ft)	20.43	11.95	0.00	0.00	t=9.37;	P=0.00	Yes
Run width (ft)	5.93	11.06	0.00	0.00	t=2.94;	P=0.01	Yes
Pool width (ft)[1]	0.00	0.00	79.43	7.47	t=58.22;	P=0.00	Yes
Pool rating[1]	0.00	0.00	4.63	0.49	t=51.78;	P=0.00	Yes
Boulder substrate (ft)[1]	1.13	1.14	5.80	2.14	0.09	0.31	Yes
Rubble substrate (ft)[1]	24.00	3.60	73.50	7.96	0.30	0.36	Yes
Gravel substrate (ft)	0.37	0.96	0.00	0.00	t=2.08;	P=0.05	No
Fine substrate (ft)	0.87	1.04	0.00	0.00	t=4.56;	P=0.00	Yes
Mean depth (ft)	1.40	4.64	3.06	1.82	0.00	1.32	No
Thalweg depth (ft)	1.06	0.31	5.56	3.54	0.14	0.29	Yes
Canopy cover (%)	1.93	3.79	1.87	4.99	0.97	1.03	No
Woody debris (%)[2]	0.00	0.00	0.00	0.00	No CI		No
Sun arc (deg)	164.57	6.29	132.63	17.23	1.16	1.33	Yes
Bank angle (deg)[1]	116.35	23.77	169.72	2.21	0.61	0.76	Yes
Veg. overhang (in)	0.25	0.49	0.57	1.79	t=0.94;	P=0.35	No
Bank cover (%)[1]	74.38	13.69	90.00	0.00	0.75	0.90	Yes
Bank alteration (%)	18.83	11.25	0.00	0.00	0.76	0.87	Yes
Bank undercut (in)	0.07	0.13	0.00	0.00	t=2.83;	P=0.01	Yes

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

Appendix B18.—Mean, standard deviation, 99% confidence interval around the ratio of means, and significance of habitat data from the channelized segment with laid-back banks and mine tailings (stream state 4/6) within Reach 9 in Silver Bow Creek and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 10.99 with 16 and 43 degrees of freedom; $P = 0.00$.

Variable	Test Mean	1SD	Reference Mean	1SD	99% CI on ratio Lower	Upper	Sign
Channel width (ft)	29.23	4.89	21.40	4.90	1.18	1.58	Yes
Wetted ch. width (ft)	20.80	2.59	15.20	4.09	1.19	1.60	Yes
Riffle width (ft)	4.47	8.57	4.37	7.10	0.04	6.01	No
Run width (ft)	16.33	7.56	4.23	6.85	2.00	20.93	Yes
Pool width (ft)	0.53	1.63	6.60	7.69	0.00	0.29	Yes
Pool rating[1]	0.47	1.43	1.70	1.93	0.00	0.97	Yes
Boulder substrate (ft)	4.03	1.73	1.30	1.53	1.82	7.75	Yes
Rubble substrate (ft)	5.40	5.15	9.07	4.43	0.30	0.97	Yes
Gravel substrate (ft)	3.27	2.10	0.10	0.54	$t=7.99$; $P=0.00$		Yes
Fine substrate (ft)	8.03	4.94	4.73	4.50	0.99	3.41	No
Mean depth (ft)	0.78	0.19	0.65	0.31	0.93	1.63	No
Thalweg depth (ft)[1]	1.39	0.39	1.18	0.53	0.92	1.54	No
Canopy cover (%)[1]	0.00	0.00	7.57	12.94	1.01	1.16	Yes
Woody debris (%)	0.33	1.26	1.76	4.43	0.99	1.04	No
Sun arc (deg)	125.47	7.86	148.80	11.51	0.80	0.89	Yes
Bank angle (deg)	155.53	13.45	136.45	28.71	1.02	1.28	Yes
Veg. overhang (in)	0.00	0.00	0.55	1.00	$t=3.00$; $P=0.01$		Yes
Bank cover (%)[1]	62.92	19.51	88.33	4.32	0.60	0.83	Yes
Bank alteration (%)	36.42	23.55	1.33	3.64	0.52	0.77	Yes
Bank undercut (in)	0.04	0.09	0.19	0.31	0.00	1.82	No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

Appendix B19.—Mean, standard deviation, and 99% confidence interval around the ratio of means, and significance of habitat data from the laid-back bank segment with mine tailings (stream state 3/6) within Reach 10 in Silver Bow Creek and its corresponding reference site. A one-sample or two-sample t-test assessed differences between means when CI could not be calculated. We considered tests with $P \leq 0.01$ as statistically significant. The multivariate Hotelling-Lawley trace = 67.03 with 13 and 46 degrees of freedom; $P = 0.00$.

Variable	Test Mean	1SD	Reference Mean	1SD	99% CI on ratio Lower	Upper	Sign
Channel width (ft)[1]	36.13	6.57	21.83	3.60	1.46	1.87	Yes
Wetted ch. width (ft)	33.20	6.64	19.37	3.62	1.49	1.96	Yes
Riffle width (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Run width (ft)[1]	32.60	7.39	0.00	0.00	t=24.14; P=0.00		Yes
Pool width (ft)	0.27	1.01	19.37	3.62	0.00	0.04	Yes
Pool rating[1]	0.33	1.26	3.77	0.63	0.00	0.26	Yes
Boulder substrate (ft)[2]	0.00	0.00	0.00	0.00	No CI		No
Rubble substrate (ft)	0.30	0.75	0.00	0.00	t=2.19; P=0.04		No
Gravel substrate (ft)	10.30	6.87	0.00	0.00	t=8.21; P=0.00		Yes
Fine substrate (ft)	22.30	6.54	19.37	3.62	0.96	1.36	No
Mean depth (ft)	0.53	0.21	1.26	0.39	0.32	0.54	Yes
Thalweg depth (ft)[1]	1.08	0.39	2.22	0.60	0.38	0.61	Yes
Canopy cover (%)[1]	4.17	8.32	0.00	0.00	0.92	1.00	No
Woody debris (%)	0.07	0.36	0.00	0.00	0.99	1.00	No
Sun arc (deg)	148.83	11.05	167.00	3.30	0.86	0.93	Yes
Bank angle (deg)	120.17	33.15	119.82	30.55	0.83	1.21	No
Veg. overhang (in)	0.68	1.34	0.00	0.00	t=2.80; P=0.01		Yes
Bank cover (%)	24.33	10.40	34.68	23.04	0.48	1.09	No
Bank alteration (%)	82.83	15.85	60.33	23.52	1.12	1.74	Yes
Bank undercut (in)	0.16	0.23	0.08	0.14	t=1.59; P=0.12		No

[1]Variable not used in multivariate analysis because it was correlated with other variables.

[2]Variable not used in multivariate analysis because it had zero variance.

APPENDIX C

Comparison of population estimates made by direct underwater observation and by the removal-depletion method of electrofishing.

Appendix C1.--Number of trout estimated by underwater observation and by the removal-depletion method of electrofishing in the Clark Fork River, 4 September 1991. This site, located about 150 m downstream of Sager Lane Bridge, was 61-m long and was selected to represent stream conditions that had little to no instream and overhead cover. The quotient S/E*100 is the ratio of snorkel to electrofishing estimates multiplied by 100.

Species	Size class (in)	Snorkel	Electrofish	S/E*100
Brown Trout	1			
	3			
	5	1	1	100
	7	3	2	150
	9	1	1	100
	11	1	1	100
	13	2	2	100
	15	2	2	100
	Total (95% CI)	10	9 (No CI)	111

Appendix C2.--Number of trout estimated by underwater observation and by the removal-depletion method of electrofishing in the Clark Fork River, 4 September 1991. This site, located about 220 m downstream of Sager Lane Bridge, was 93-m long and was selected to represent stream conditions with abundant instream and overhead cover. The quotient S/E*100 is the ratio of snorkel to electrofishing estimates multiplied by 100.

Species	Size class (in)	Snorkel	Electrofish	S/E*100
Brown Trout	1			
	3	6	1	600
	5	4	3	133
	7	17	19	90
	9	9	10	90
	11	5	5	100
	13	5	3	167
	15	5	6	80
	17	3	4	80
	19	1	1	100
	Total (95% CI)	55	54 (49-59)	102

Appendix C3.--Number of trout estimated by underwater observation and by the removal-depletion method of electrofishing in Rock Creek, 5 September 1991. This site, located at about river mile 2.0, was 100-m long and was selected to represent stream conditions that had abundant instream and overhead cover. The quotient S/E*100 is the ratio of snorkel to electrofishing estimates multiplied by 100.

Species	Size class (in)	Snorkel	Electrofish	S/E*100
Brown Trout	1			
	3	49	52	94
	5	8	5	160
	7	7	9	78
	9	5	3	167
	11	1	1	100
	13	2	2	100
	15	1	1	100
	Total (95% CI)	73	76(71-81)	96
Rainbow Trout	1			
	3	9	11	82
	5	1	1	100
	Total (95% CI)	10	13(8-18)	77

Appendix C4.--Number of brown trout estimated by underwater observation and by the removal-depletion method of electrofishing in the Big Hole River, 9 September 1991. This site, located about 4.25 miles downstream from the town of Glen, was 65-m long and was selected to represent stream conditions that had abundant instream and overhead cover. The quotient S/E*100 is the ratio of snorkel to electrofishing estimates multiplied by 100.

Species	Size class (in)	Snorkel	Electrofish	S/E*100
Brown Trout	1			
	3	14	9	155
	5	7	1	700
	7	6	8	75
	9	5	5	100
	11	2	1	200
	13	2	3	67
	15	3	3	100
	17	2	2	100
	19	3	3	100
	Total (95% CI)	44	35(33-37)	126

Appendix C5.--Number of trout estimated by underwater observation and by the removal-depletion method of electrofishing in Flint Creek, 6 September 1991. This site, located 1.3 miles upstream from the Hall Bridge, was 100-m long and was selected to represent stream conditions that had abundant instream and overhead cover. The quotient S/E*100 is the ratio of snorkel to electrofishing estimates multiplied by 100.

Species	Size class (in)	Snorkel	Electrofish	S/E*100
Brown Trout	1			
	3	13	11	118
	5	3	4	75
	7	34	40	85
	9	16	15	107
	11	15	16	94
	13	10	9	111
	15	6	7	86
	17	1	2	50
	19	1	0	---
	Total (95% CI)	99	105(103-107)	94

Appendix C6.--Number of trout estimated by underwater observation and by the removal-depletion method of electrofishing in Bison Creek, 7 September 1991. This site, located about 150 m downstream from the confluence of Wilder Gulch, was 100-m long and was selected to represent stream conditions that had abundant instream and overhead cover. The quotient S/E*100 is the ratio of snorkel to electrofishing estimates multiplied by 100.

Species	Size class (in)	Snorkel	Electrofish	S/E*100
Brook Trout	1			
	3	3	4	75
	5	5	3	167
	7	3	3	100
	Total (95% CI)	11	10(9-11)	110
Rainbow Trout	1			
	3	7	3	233
	5	54	50	108
	7	19	19	100
	9	5	6	83
	11	2	2	100
	13	1	1	100
	Total (95% CI)	88	84(79-89)	105

APPENDIX D

Length-weight relationships for trout in test and reference streams.

Regression of Weight on Length
Brown Trout in the Clark Fork

$$W=0.028 + 0.00038L^3$$
$$r^2=0.98 \quad P=0.00$$
$$SE=4.26E\text{-}6$$

Weight (lbs.)

Length (in.)^3
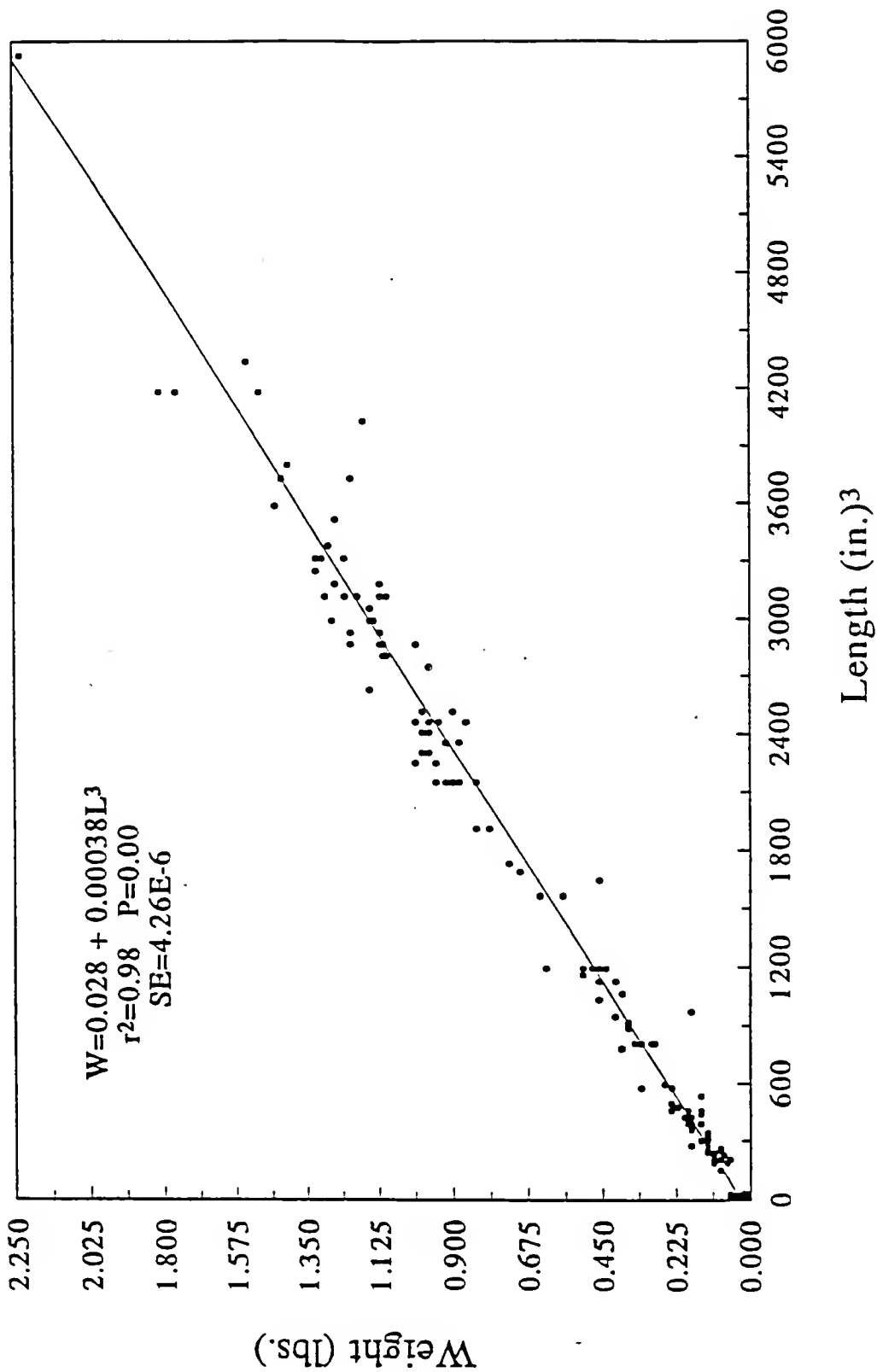
Appendix D1.--Length-weight relationship for brown trout in the Clark Fork River, 1991.

Regression of Weight on Length
Rainbow Trout in the Clark Fork

$W=0.031 + 0.00037L^3$
$r^2=0.97$ $P=0.00$
$SE=4.28E-6$

Weight (lbs.)

Length (in.)^3
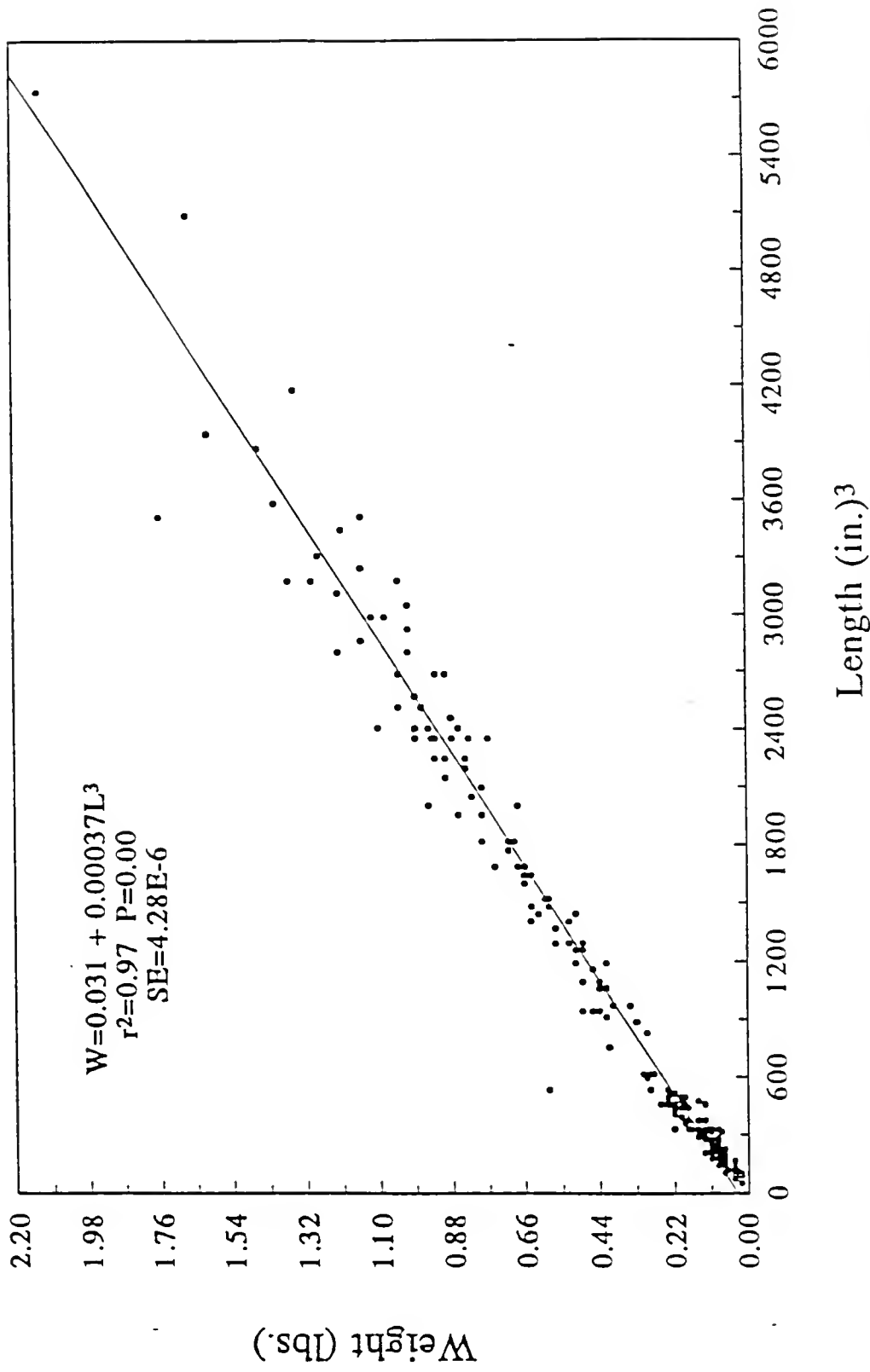
Appendix D2.--Length-weight relationship for rainbow trout in the Clark Fork River, 1991.

Regression of Weight on Length
Brown Trout in Rock Creek

$W=-0.0085 + 0.00036L^3$
$r^2=0.99$ $P=0.00$
$SE=3.65E-6$

Weight (lbs.)

Length (in.)^3

Appendix D3.--Length-weight relationship for brown trout in Rock Creek, 1991.

Regression of Weight on Length
Rainbow Trout in Rock Creek

$W=0.00027L^{3.12}$ (antilog form)
$r^2=0.98$ P=0.00
SE=3.49E-2

Log Weight (lbs.)

Log Length (in.)
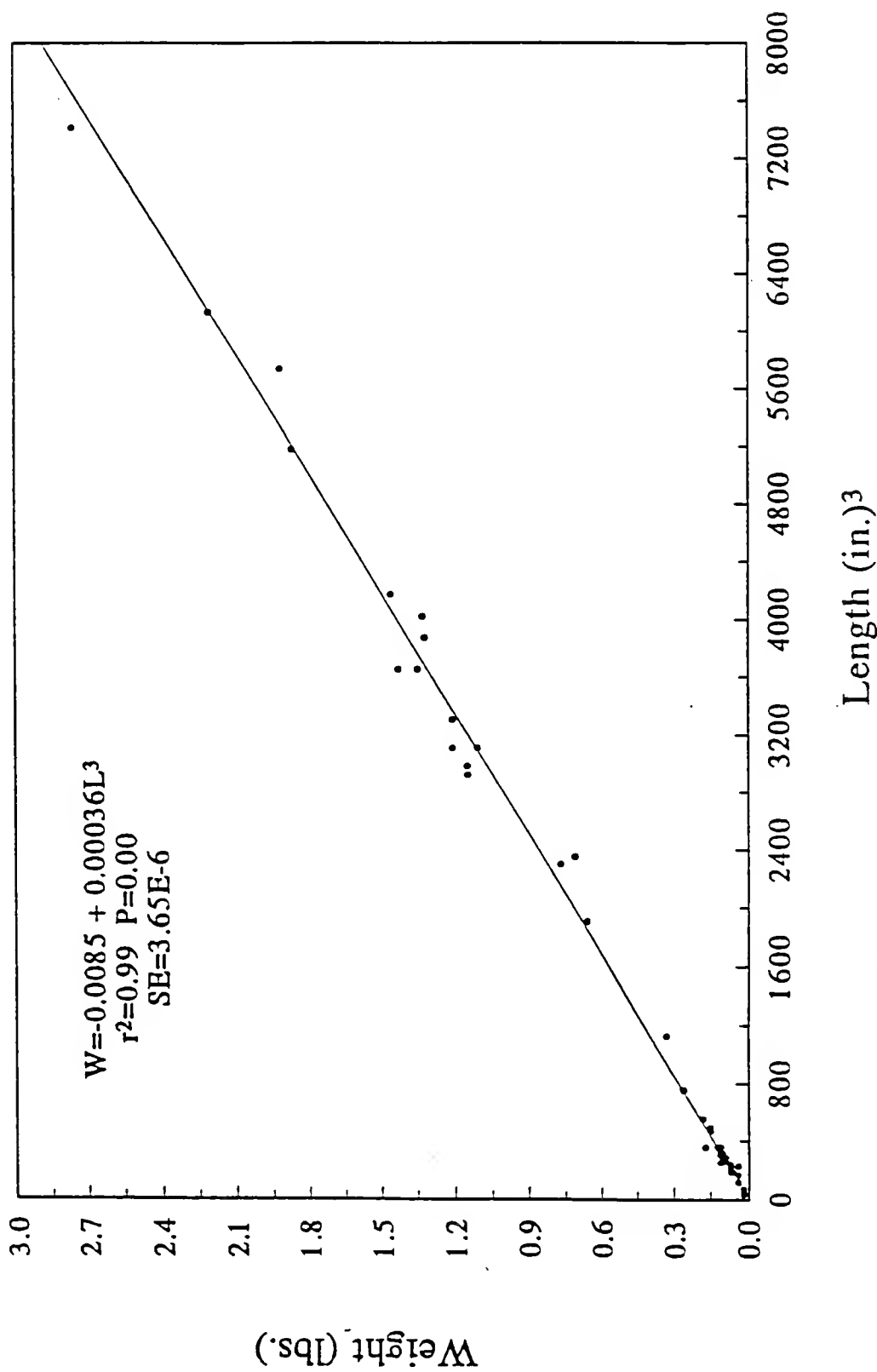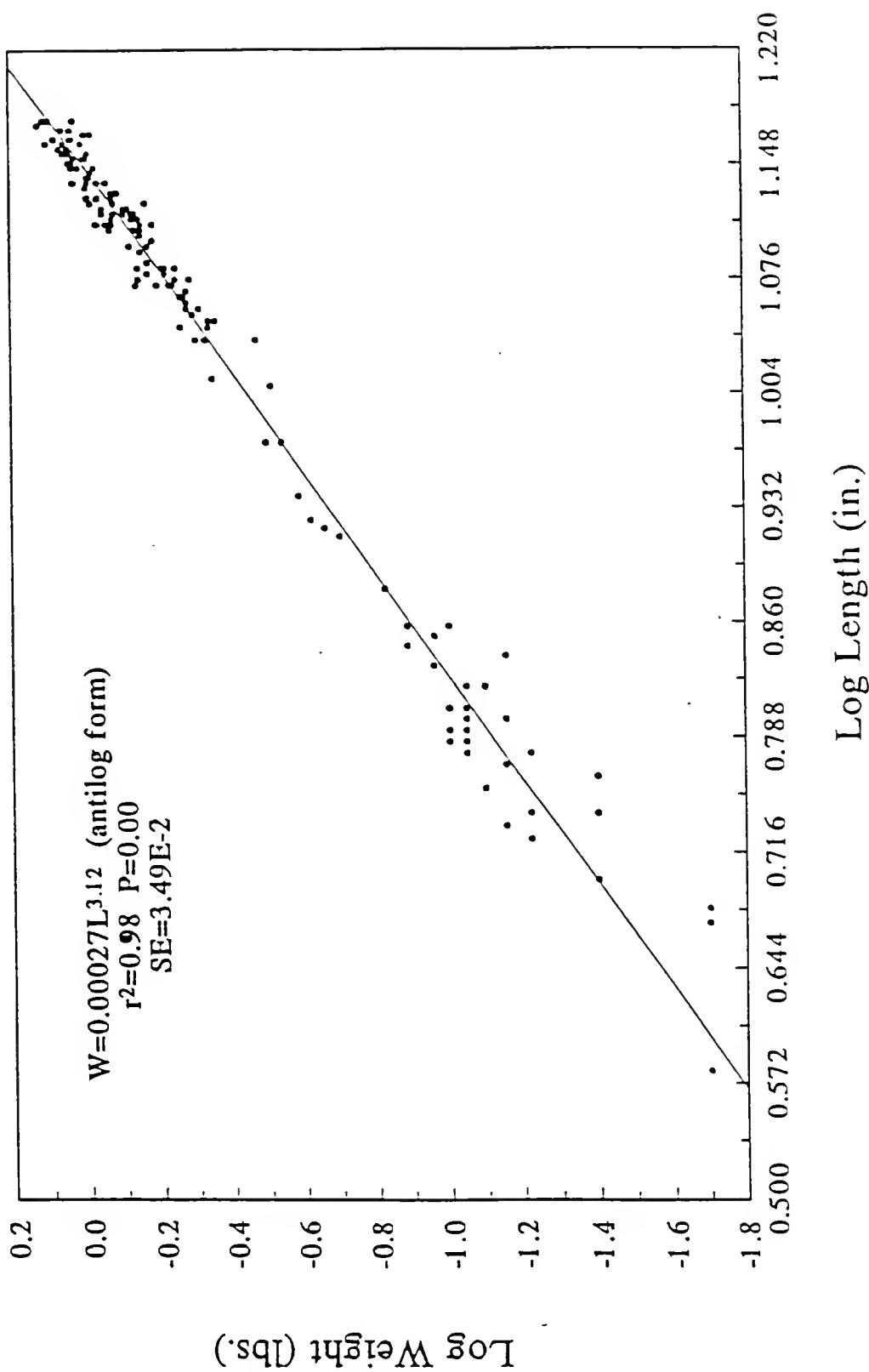
Appendix D4.--Length-weight relationship for rainbow trout in Rock Creek, 1991.

Regression of Weight on Length
Brown Trout in the Big Hole

$W=0.007 + 0.00037L^3$
$r^2=0.99$ $P=0.00$
$SE=3.91E-6$

Weight (lbs.)

Length (in.)^3
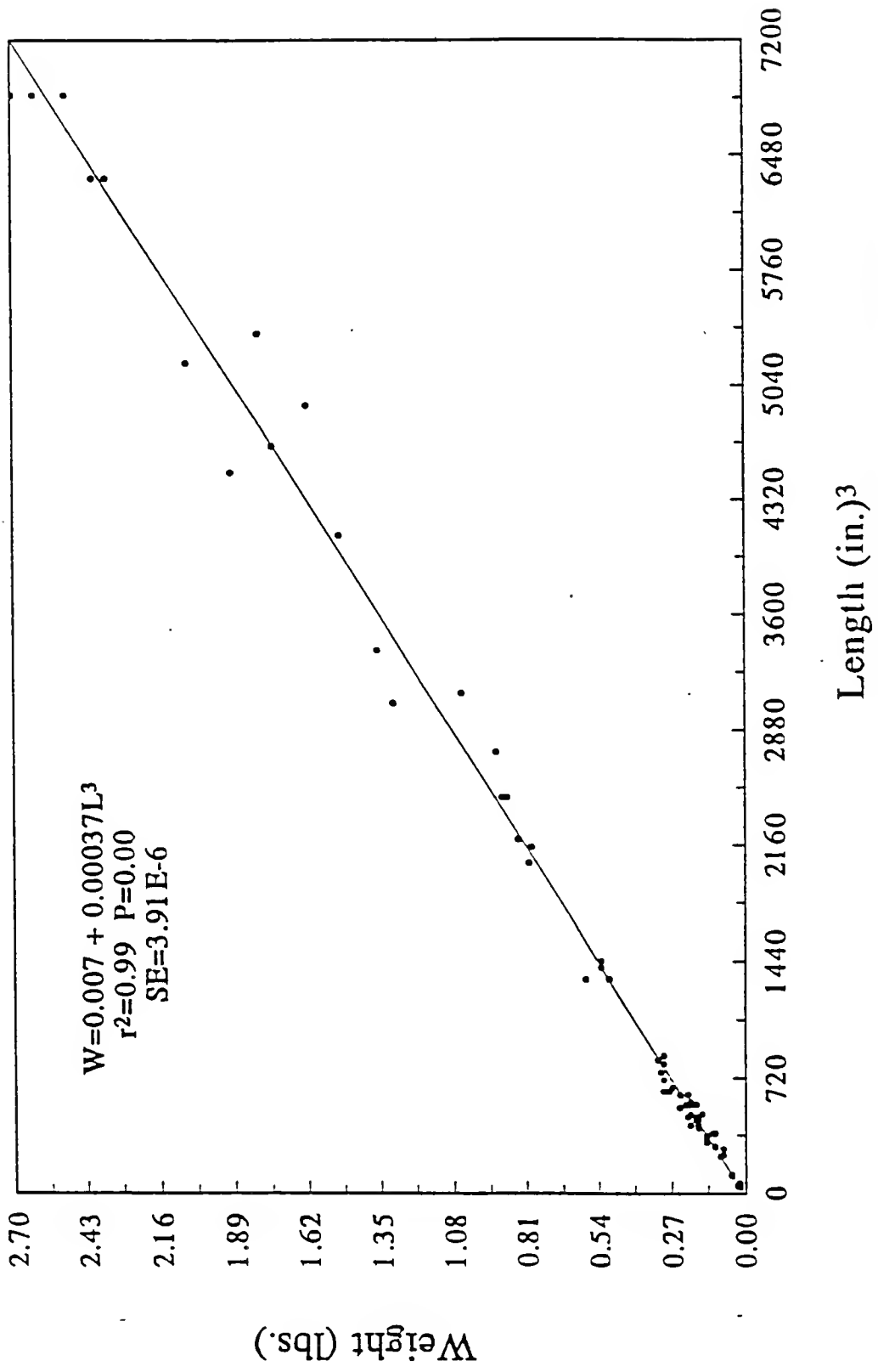
Appendix D5.--Length-weight relationship for brown trout in the Big Hole River, 1991.

Regression of Weight on Length
Rainbow Trout in the Big Hole

$W = 0.00059L^{2.8}$ (antilog form)
$r^2 = 0.97$ $P = 0.00$
$SE = 3.82E\text{-}2$

Log Weight (lbs.)

Log Length (in.)
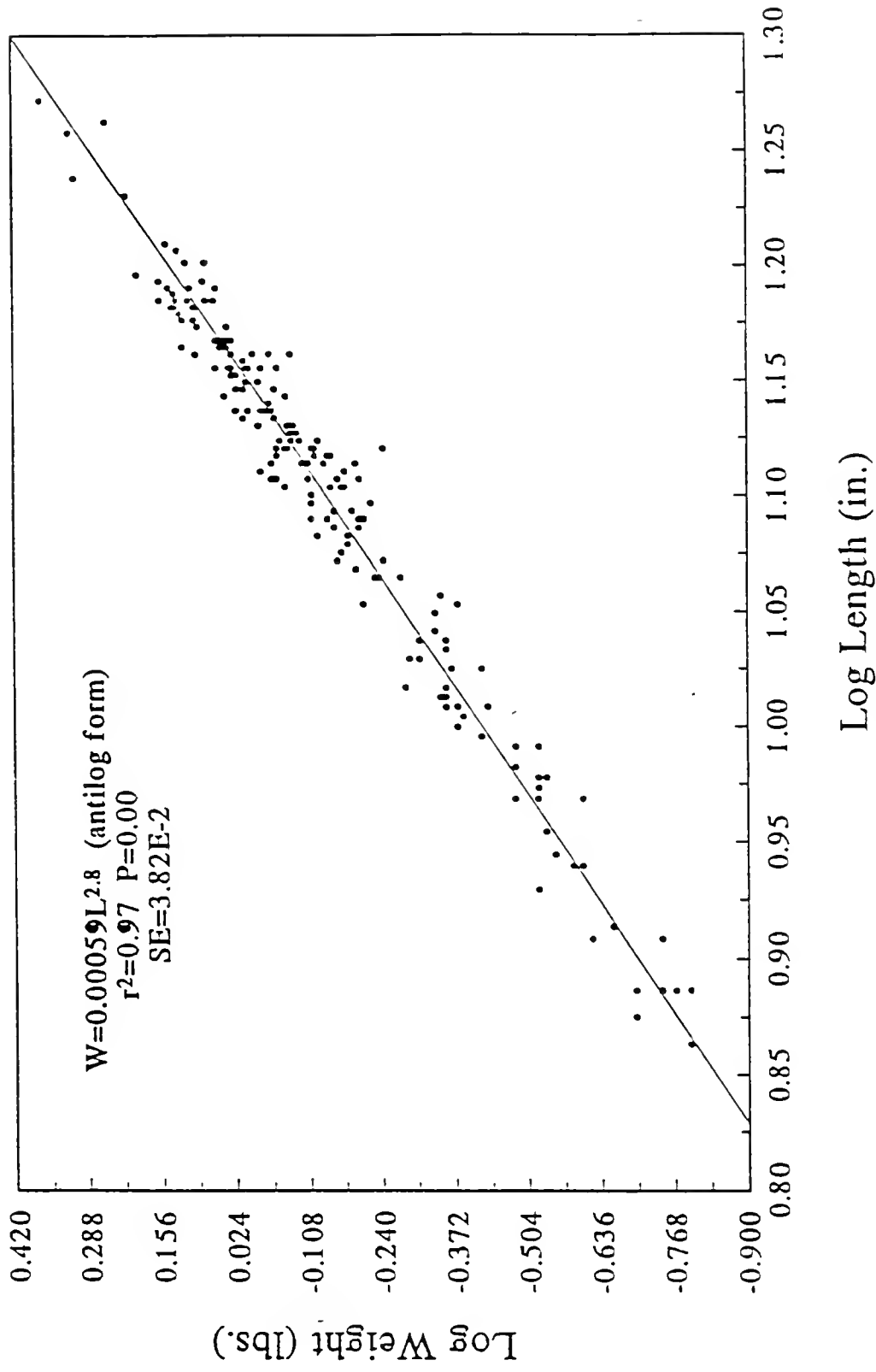
Appendix D6.--Length-weight relationship for rainbow trout in the Big Hole River, 1991.

Regression of Weight on Length
Brown Trout in Flint Creek

$W = 0.019 + 0.00036L^3$
$r^2 = 0.99 \quad P = 0.00$
$SE = 3.98E-6$

Weight (lbs.)

Length (in.)^3

Appendix D7.--Length-weight relationship for brown trout in Flint Creek, 1991.
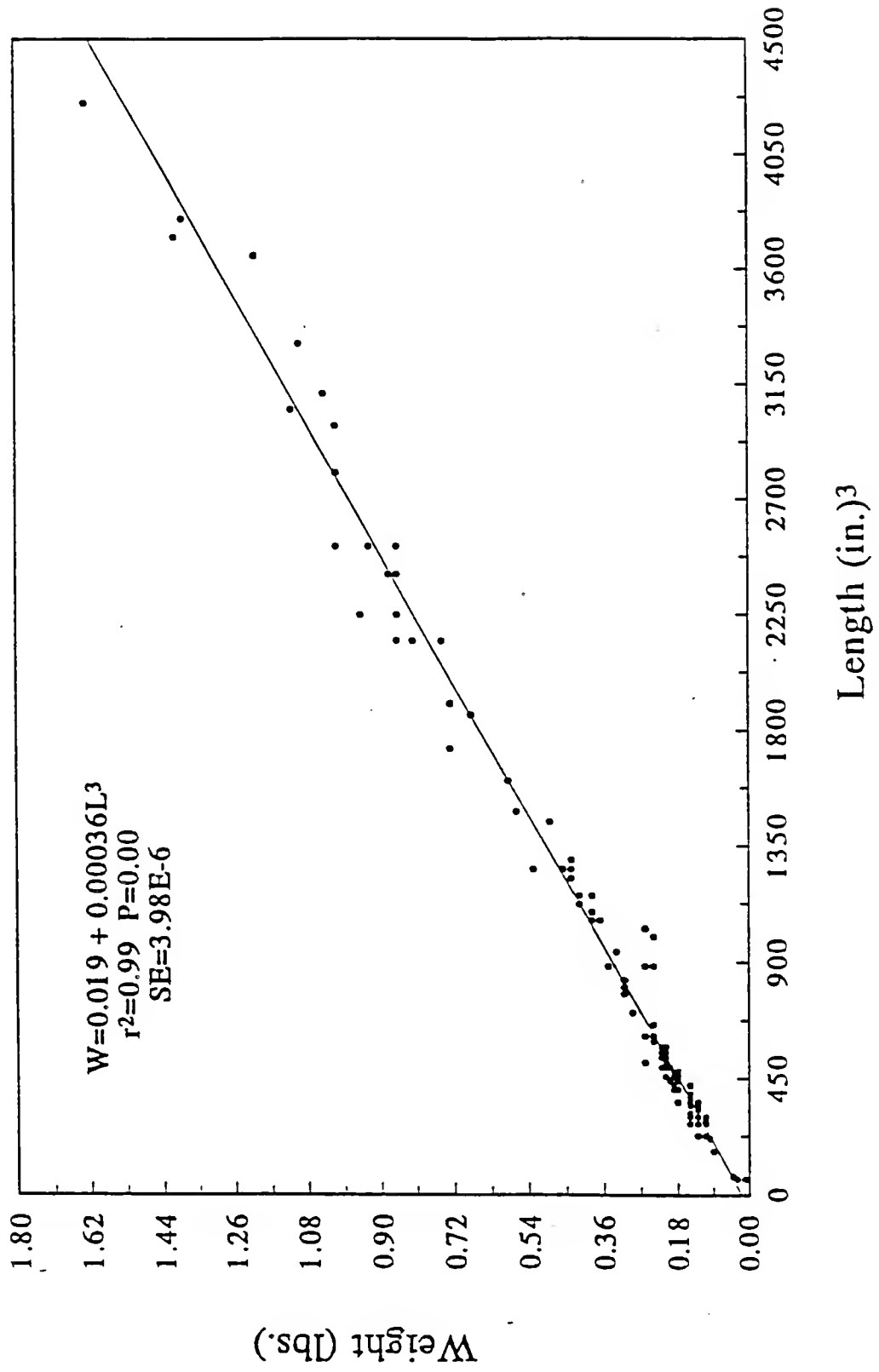
Regression of Weight on Length
Brook Trout in Bison Creek

$W = 0.013 + 0.00032L^3$
$r^2 = 0.74$ $P = 0.00$
$SE = 3.49E\text{-}5$

Weight (lbs.)

Length (in.)^3
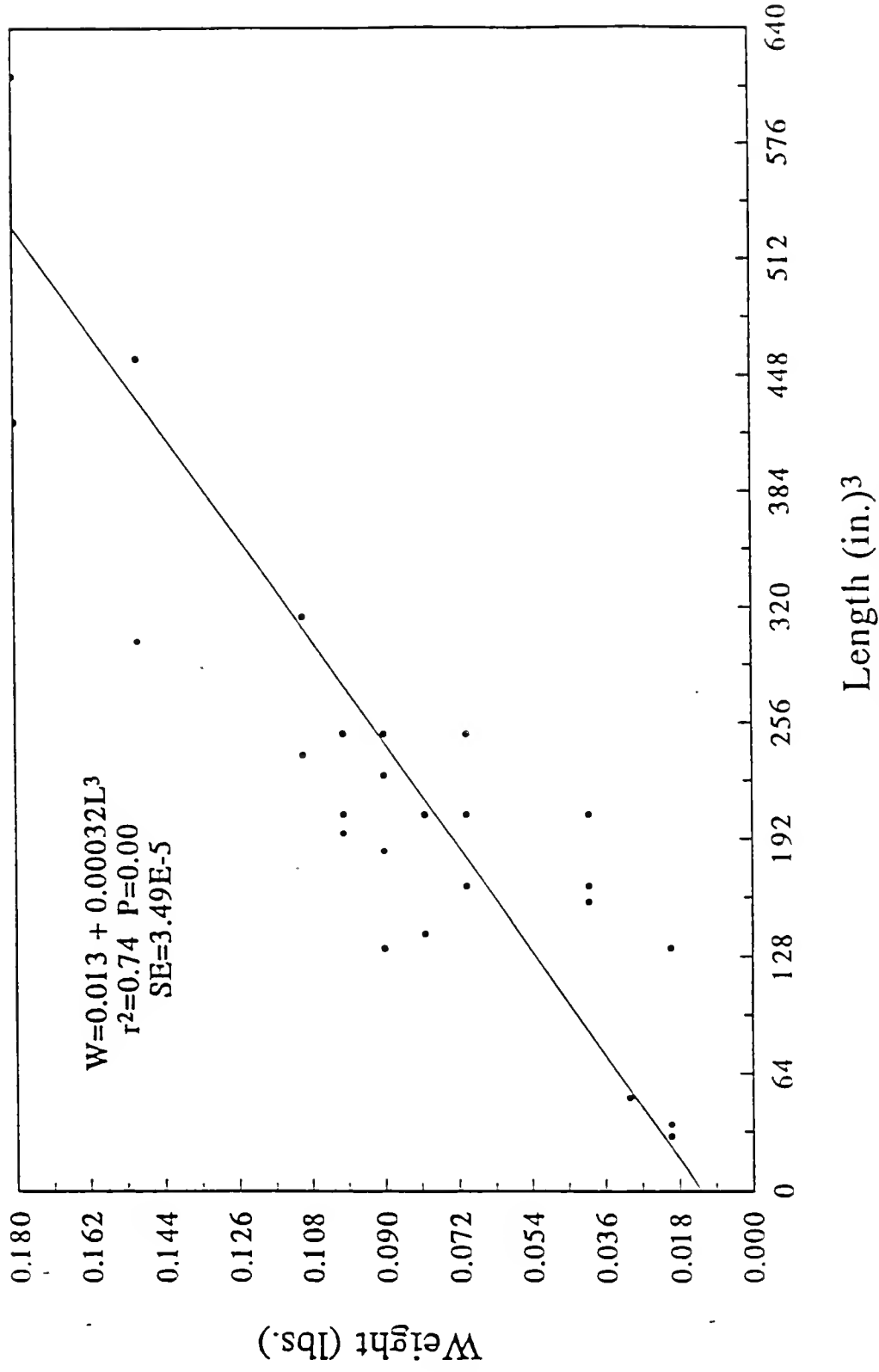
Appendix D8.--Length-weight relationship for brook trout in Bison Creek, 1991.

Regression of Weight on Length
Rainbow Trout in Bison Creek

W=0.0066 + 0.0004L³
r²=0.97 P=0.00
SE=7.3E-6

Weight (lbs.)

Length (in.)³
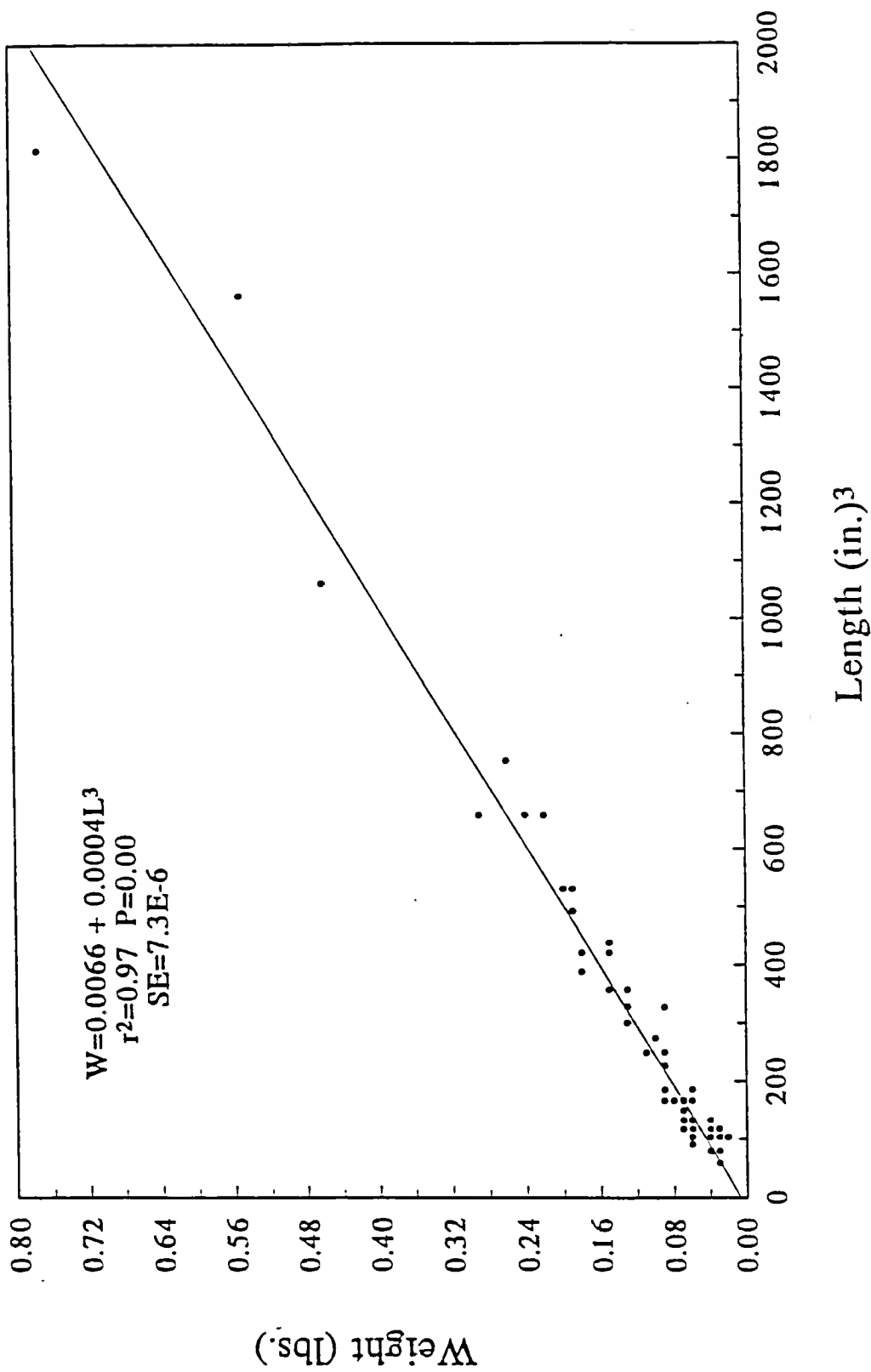
Appendix D9.--Length-weight relationship for rainbow trout in Bison Creek, 1991.

Regression of Weight on Length
Brown Trout in the Ruby River

$W=0.00047L^{2.92}$ (antilog form)
$r^2=0.98$ $P=0.00$
$SE=2.43E-2$

Log Length (in.)

Log Weight (lbs.)
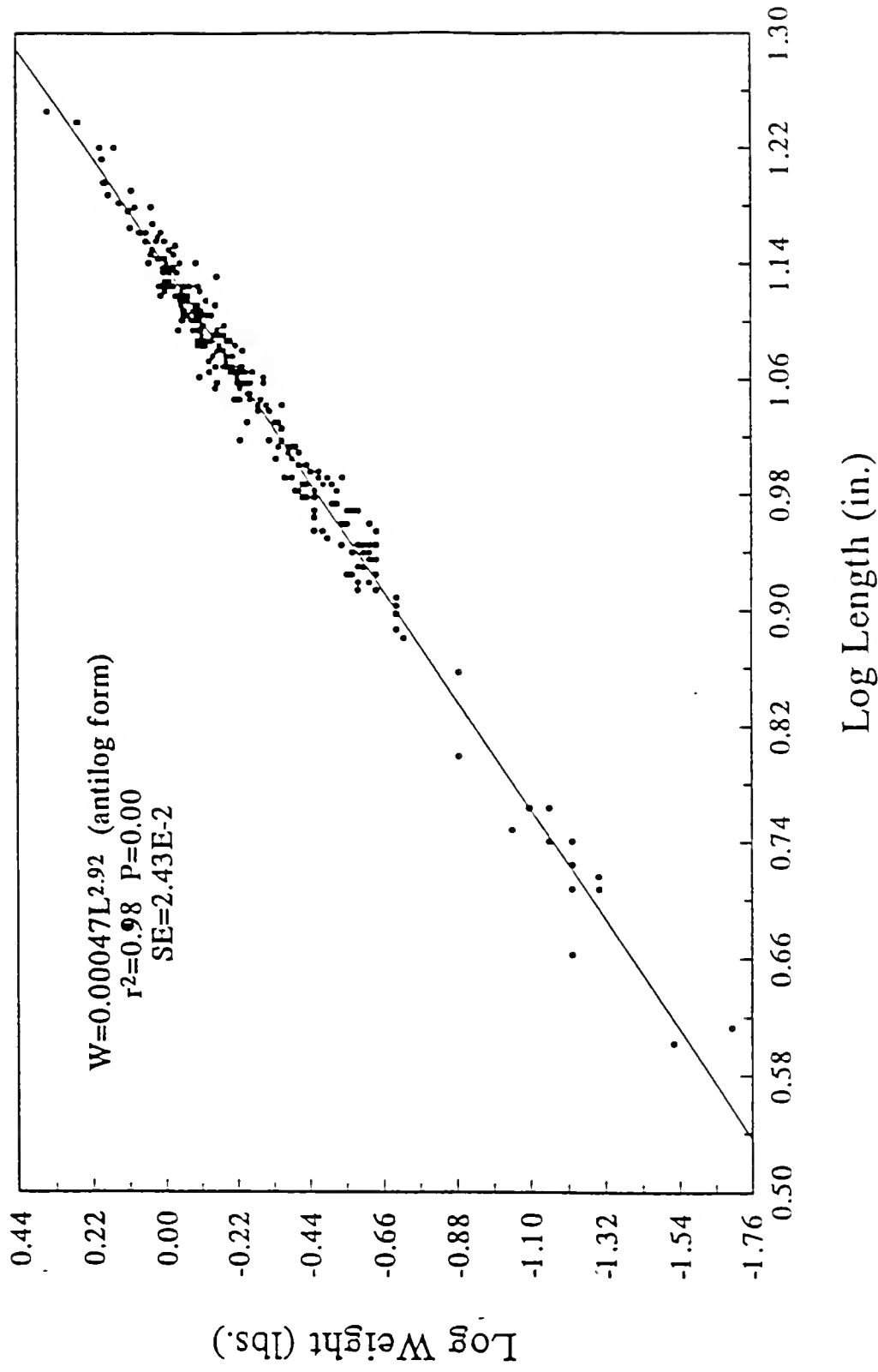
Appendix D10.--Length-weight relationship for brown trout in the Ruby River, 1991.

Regression of Weight on Length
Rainbow Trout in the Ruby River

$$W=0.00082L^{2.68} \text{ (antilog form)}$$
$$r^2=0.97 \quad P=0.00$$
$$SE=3.52E\text{-}2$$

Log Weight (lbs.)

Log Length (in.)

Appendix D11.--Length-weight relationship for rainbow trout in the Ruby River, 1991.
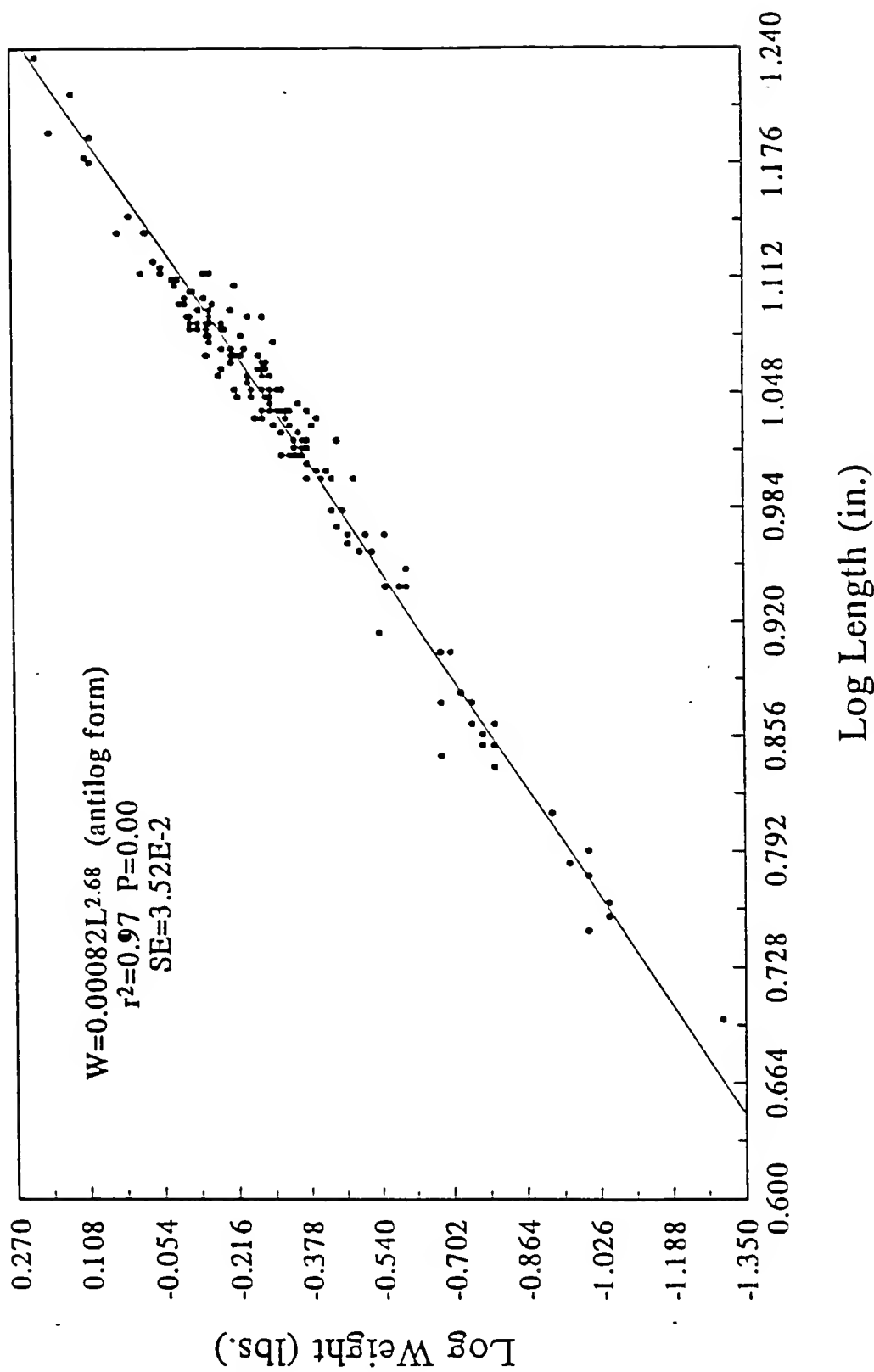
APPENDIX E

Weighted usable area (WUA m^2/site) for trout in test and reference states.

Appendix E1.--Weighted usable area (WUA m^2/site) for trout and whitefish in stream states in Reach 1 in the Clark Fork River and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

Species	Age class	State 7/3 (WUA m^2/site)		State 4/5 (WUA m^2/site)		State 3/5 (WUA m^2/site)	
		Test	Reference	Test	Reference	Test	Reference
Brown	Fry	255.2	257.0	160.4	129.8	426.2	257.0
	Juvenile	750.0	884.8	576.7	495.1	1316.6	884.8
	Adult	637.1	724.8	491.8	439.4	1106.4	724.8
Rainbow	Fry	282.0	371.3	374.2	254.0	714.3	371.3
	Juvenile	337.9	627.6	707.8	363.3	1104.2	627.6
	Adult	1430.2	1660.9	1866.2	1233.0	3160.0	1660.9
Brook	Fry	163.7	37.8	32.7	22.3	145.4	37.8
	Juvenile	251.5	53.2	65.9	25.6	135.0	53.2
	Adult	526.7	843.7	783.1	534.5	1504.9	843.7

Appendix E2.--Weighted usable area (WUA m²/site) for trout and whitefish in stream states in Reach 2 in the Clark Fork River and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

Species	Age class	State 3 (WUA m²/site)		State 4 (WUA m²/site)		State 2 (WUA m²/site)	
		Test	Reference	Test	Reference	Test	Reference
Brown	Fry	154.5	129.8	172.8	277.2	281.4	165.2
	Juvenile	396.7	495.1	535.1	980.6	626.4	546.5
	Adult	354.2	439.4	462.5	772.0	494.4	482.8
Rainbow	Fry	184.5	254.0	243.7	518.5	422.1	253.2
	Juvenile	166.4	363.3	257.5	502.0	359.9	213.7
	Adult	660.5	1233.0	936.6	1490.4	1209.6	1336.4
Brook	Fry	257.6	22.3	64.6	19.2	175.4	73.2
	Juvenile	195.1	25.6	58.6	29.0	68.9	33.3
	Adult	329.3	534.5	470.8	787.6	673.3	483.6

Appendix E3.—Weighted usable area (WUA m^2/site) for trout and whitefish in stream states in Reach 3 in the Clark Fork River and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

Species	Age class	State 4 (WUA m^2/site)		State 3 (WUA m^2/site)	
		Test	Reference	Test	Reference
Brown Trout	Fry	974.2	172.3	276.5	158.6
	Juvenile	1660.3	589.3	631.9	535.4
	Adult	1116.4	595.3	434.5	456.2
Rainbow Trout	Fry	695.5	1217.6	254.9	380.5
	Juvenile	645.8	784.7	195.7	528.6
	Adult	2700.5	2422.3	966.6	1911.7
Brook Trout	Fry	218.7	0.3	238.7	0.0
	Juvenile	96.6	0.0	164.3	0.0
	Adult	1156.1	1783.6	500.4	735.5

Appendix E4.--Weighted usable area (WUA m²/site) for trout and whitefish in stream states in Reach 4 and 5 in the Clark Fork River and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

| Species | Age class | Reach 4 | | | | Reach 5 | |
| | | State 3 (WUA m²/site) | | State 4 (WUA m²/site) | | State 4 (WUA m²/site) | |
		Test	Reference	Test	Reference	Test	Reference
Brown	Fry	262.5	460.3	553.2	172.3	814.3	322.9
	Juvenile	559.6	1351.1	1135.7	589.3	1189.9	964.5
	Adult	418.1	1053.5	860.5	595.3	766.6	766.6
Rainbow	Fry	227.4	696.3	650.5	1217.6	507.6	1074.6
	Juvenile	208.8	936.3	601.0	784.7	588.0	865.5
	Adult	859.4	2447.6	2125.6	2422.3	1688.2	3372.6
Brook	Fry	120.1	39.6	69.5	0.3	252.9	0.0
	Juvenile	72.7	13.6	42.3	0.0	184.5	0.0
	Adult	407.4	1245.1	1005.5	1783.6	921.7	1629.3

Appendix E5.--Weighted usable area (WUA m²/site) for trout and whitefish in stream states in Reach 6 in the Clark Fork River and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

Species	Age class	State 2d (WUA m²/site)		State 4 (WUA m²/site)	
		Test	Reference	Test	Reference
Brown Trout	Fry	555.4	36.7	465.4	89.6
	Juvenile	718.5	107.2	777.2	258.5
	Adult	576.8	100.4	744.0	223.9
Rainbow Trout	Fry	138.6	401.4	564.3	351.2
	Juvenile	157.9	322.3	275.8	227.5
	Adult	1009.4	473.4	948.4	378.4
Brook Trout	Fry	263.2	13.2	893.3	64.5
	Juvenile	193.8	5.5	787.0	65.1
	Adult	252.0	421.4	715.0	511.3

Appendix E5.--Concluded.

Species	Age class	State 2u (WUA m^2/site)		State 1/2 (WUA m^2/site)	
		Test	Reference	Test	Reference
Brown Trout	Fry	203.2	36.7	229.7	389.4
	Juvenile	368.9	107.2	297.2	556.1
	Adult	379.2	100.4	165.6	420.0
Rainbow Trout	Fry	516.8	401.4	59.1	55.2
	Juvenile	235.8	322.3	75.5	85.2
	Adult	575.7	473.4	407.1	767.2
Brook Trout	Fry	550.4	13.2	93.7	104.0
	Juvenile	392.3	5.5	77.7	103.4
	Adult	682.0	421.4	166.1	130.0

Appendix E6.--Weighted usable area (WUA m^2/site) for trout and whitefish in stream states in Reach 7 in Silver Bow Creek and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

Species	Age class	State 2 (WUA m^2/site)		State 4/6 (WUA m^2/site)	
		Test	Reference	Test	Reference
Brown Trout	Fry	226.3	193.7	42.1	176.4
	Juvenile	268.4	484.9	114.9	690.1
	Adult	153.6	406.7	103.3	618.3
Rainbow Trout	Fry	48.7	244.8	41.8	853.0
	Juvenile	49.7	235.8	42.6	1907.8
	Adult	379.6	980.6	290.5	2116.9
Brook Trout	Fry	31.6	92.1	29.4	0.1
	Juvenile	20.5	107.8	8.7	0.0
	Adult	100.4	365.4	92.6	2403.5

Appendix E7.--Weighted usable area (WUA m²/site) for trout and whitefish in stream states in Reach 9 and 10 in Silver Bow Creek and their corresponding reference sites. We consider a fish less than 2 inches a fry, 2 to 8 inches a juvenile, and larger than 8 inches an adult.

Species	Age class	Reach 9 - State 4/6 (WUA m²/site)		Reach 10 - State 3/6 (WUA m²/site)	
		Test	Reference	Test	Reference
Brown Trout	Fry	109.4	41.5	130.3	96.1
	Juvenile	193.0	72.9	172.9	216.1
	Adult	162.1	96.6	199.1	217.7
Rainbow Trout	Fry	71.6	159.9	20.5	27.6
	Juvenile	57.4	71.9	19.8	51.8
	Adult	319.3	144.6	317.3	275.9
Brook Trout	Fry	48.6	88.2	79.7	41.7
	Juvenile	18.5	52.4	47.2	76.7
	Adult	101.9	218.8	18.5	56.8

APPENDIX F

Mean densities and biomasses of trout in test and reference state types.

Appendix F1.--Densities and biomasses of all trout that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	34.5	210.9	42.4	72.8	8.8	41.6	6.6	11.2
1	4/5	38.0	214.8	17.0	88.2	14.0	46.6	5.5	13.3
1	3/5	81.9	148.1	27.0	50.7	27.5	28.1	6.5	7.4
2	3	29.9	241.8	52.5	158.8	6.3	53.3	6.8	21.6
2	4	6.5	193.1	5.5	128.2	3.9	52.2	3.2	21.8
2	2	4.5	224.1	4.0	266.4	1.0	41.2	1.0	32.5
3	4	4.3	33.9	1.2	34.7	1.3	9.0	0.4	9.0
3	3	7.0	50.3	5.1	28.7	2.8	25.3	2.2	14.9
4	3	11.6	41.2	5.0	15.8	4.2	18.5	1.8	6.6
4	4	5.8	19.2	2.3	16.7	2.7	4.2	1.2	3.0
5	4	19.8	26.4	4.7	13.5	8.7	6.9	2.2	3.5
6	2d	10.1	458.8	4.1	375.3	4.1	126.1	1.7	105.4
6	4	20.6	116.3	4.6	49.7	4.1	22.3	0.9	9.6
6	2u	2.1	794.1	10.9	656.1	0.3	271.2	1.4	226.3
6	1/2	583.7	773.7	1130.0	955.7	126.9	234.4	333.4	300.8
7	2	0.0	57.4	0.0	18.4	0.0	12.9	0.0	4.0
7	4/6	0.0	34.2	0.0	7.8	0.0	8.2	0.0	2.7
9	4/6	0.0	392.6	0.0	319.1	0.0	69.7	0.0	45.4
10	3/6	0.0	558.3	0.0	220.7	0.0	26.8	0.0	11.0

Appendix F2.--Densities and biomasses of all juvenile trout (2 to 8 inches) that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m^2/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	25.4	157.2	38.9	60.9	2.9	7.2	4.4	2.7
1	4/5	12.5	151.6	9.3	70.4	1.6	8.6	1.2	3.9
1	3/5	45.2	103.7	20.6	40.9	5.2	5.1	2.4	2.1
2	3	24.4	164.8	48.7	133.2	1.9	8.3	3.7	6.7
2	4	1.1	127.4	2.0	107.7	0.1	10.2	0.2	7.7
2	2	2.5	178.2	2.0	235.1	0.3	8.0	0.3	10.3
3	4	1.4	19.3	0.3	23.4	0.1	1.8	0.1	2.1
3	3	2.8	22.8	1.6	15.5	0.4	2.3	0.2	1.6
4	3	5.8	21.1	2.3	10.0	0.9	1.8	0.4	0.9
4	4	3.2	11.7	1.1	12.2	0.5	1.1	0.2	1.1
5	4	7.0	14.9	1.3	8.5	0.7	1.4	0.1	0.7
6	2d	3.0	244.1	1.1	193.5	0.3	25.8	0.1	20.4
6	4	14.7	83.7	3.3	35.4	1.6	7.2	0.4	3.0
6	2u	2.1	388.2	10.9	307.8	0.3	41.8	1.4	33.1
6	1/2	416.3	541.9	662.6	653.3	16.5	23.6	26.1	25.7
7	2	0.0	38.9	0.0	13.0	0.0	2.5	0.0	0.8
7	4/6	0.0	22.5	0.0	4.7	0.0	1.1	0.0	0.2
9	4/6	0.0	259.2	0.0	260.2	0.0	21.3	0.0	25.5
10	3/6	0.0	541.7	0.0	211.9	0.0	23.3	0.0	9.1

Appendix F3.--Densities and biomasses of all adult trout (larger than 8 inches) that we observed in test and reference stream, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	9.1	53.6	3.6	12.0	5.9	34.4	2.2	8.4
1	4/5	25.5	59.4	7.8	14.4	12.5	38.1	4.3	9.4
1	3/5	36.2	44.4	6.4	9.8	22.2	23.0	4.2	5.4
2	3	5.5	75.4	3.7	23.6	4.3	45.1	3.1	14.8
2	4	5.4	65.7	3.5	20.5	3.7	43.1	3.0	14.1
2	2	2.0	44.3	2.0	27.8	0.7	33.1	0.7	22.1
3	4	2.8	14.7	0.9	11.3	1.2	7.2	0.4	6.9
3	3	4.2	27.6	3.5	13.1	2.4	23.1	2.0	13.4
4	3	5.9	20.1	2.7	5.7	3.3	16.7	1.5	5.7
4	4	2.6	7.5	1.2	4.5	2.2	3.1	1.0	1.9
5	4	12.8	11.5	3.4	5.0	8.1	5.6	2.1	2.7
6	2d	7.1	214.7	3.0	181.8	3.7	100.4	1.6	85.0
6	4	5.9	32.6	1.3	14.3	2.5	15.0	0.6	6.7
6	2u	0.0	400.0	0.0	334.7	0.0	229.3	0.0	193.0
6	1/2	167.4	231.8	467.4	302.4	110.4	210.8	307.4	275.1
7	2	0.0	18.5	0.0	5.4	0.0	10.4	0.0	3.2
7	4/6	0.0	11.7	0.0	3.1	0.0	7.1	0.0	2.4
9	4/6	0.0	111.1	0.0	49.5	0.0	43.9	0.0	19.8
10	3/6	0.0	16.7	0.0	8.8	0.0	3.6	0.0	1.9

Appendix F4.--Densities and biomasses of all brown trout that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	1.0	80.0	0.7	26.0	0.2	15.9	0.2	5.8
1	4/5	1.4	57.8	1.4	21.9	1.1	10.8	1.2	4.6
1	3/5	3.0	41.7	1.3	13.6	2.4	9.2	1.1	3.3
2	3	8.5	31.1	8.9	20.1	1.6	9.2	1.8	6.3
2	4	1.6	14.3	1.7	7.2	2.2	6.0	2.2	3.3
2	2	4.5	127.0	4.0	104.6	1.0	24.5	1.0	21.6
3	4	4.3	10.9	1.2	16.1	1.3	4.4	0.4	6.5
3	3	7.0	19.3	5.1	14.6	2.8	15.6	2.2	12.3
4	3	10.5	13.6	4.7	5.6	3.3	8.9	1.6	4.2
4	4	5.8	6.8	2.3	8.5	2.7	1.4	1.2	1.7
5	4	18.6	14.9	4.5	8.7	7.7	4.3	2.1	2.8
6	2d	10.1	458.8	4.1	375.3	4.1	126.1	1.8	105.4
6	4	20.6	100.0	4.6	43.3	4.1	19.1	0.9	8.6
6	2u	2.1	788.2	10.9	655.1	0.3	269.4	1.4	226.0
6	1/2	579.4	751.8	1114.3	814.9	124.2	233.9	330.9	297.9
7	2	0.0	46.3	0.0	13.7	0.0	11.0	0.0	3.5
7	4/6	0.0	8.3	0.0	3.8	0.0	4.4	0.0	2.1
9	4/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	3/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix F5.--Densities and biomasses of juvenile brown trout (2 to 8 inches) that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m^2/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	0.5	63.6	0.3	19.8	0.1	3.2	0.1	1.0
1	4/5	0.5	46.1	0.4	16.7	0.1	2.8	0.1	1.0
1	3/5	0.5	29.6	0.2	9.0	0.1	1.3	0.1	0.4
2	3	7.9	19.7	8.2	12.1	0.8	1.0	0.8	0.6
2	4	0.0	8.0	0.0	3.6	0.0	0.8	0.0	0.4
2	2	2.5	102.9	2.0	81.9	0.3	4.8	0.3	3.8
3	4	1.4	5.5	0.3	9.1	0.1	0.5	0.1	0.7
3	3	2.8	6.2	1.6	4.1	0.4	0.7	0.2	0.5
4	3	5.9	7.0	2.3	2.6	0.9	0.5	0.4	0.2
4	4	3.2	4.5	1.1	5.5	0.5	0.4	0.2	0.4
5	4	7.0	8.2	1.3	4.4	0.7	0.8	0.1	0.4
6	2d	3.0	244.1	1.1	193.5	0.3	25.8	0.1	20.4
6	4	14.7	74.4	3.3	31.0	1.6	6.2	0.4	2.5
6	2u	2.1	388.2	10.9	307.8	0.3	41.8	1.4	33.1
6	1/2	414.2	520.1	649.4	512.5	16.5	23.1	25.8	22.8
7	2	0.0	31.5	0.0	8.8	0.0	2.1	0.0	0.6
7	4/6	0.0	4.2	0.0	1.8	0.0	0.2	0.0	0.1
9	4/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	3/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix F6.--Densities and biomasses of adult brown trout (larger than 8 inches) that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	0.5	16.4	0.4	6.3	0.1	12.7	0.1	4.8
1	4/5	1.0	11.7	1.0	5.2	1.1	8.1	1.1	3.6
1	3/5	2.5	12.0	1.1	4.5	2.3	7.9	1.1	2.9
2	3	0.6	11.5	0.7	8.0	0.8	8.3	0.9	5.7
2	4	1.6	6.3	1.7	3.6	2.2	5.3	2.2	3.0
2	2	2.0	23.6	2.0	21.2	0.7	19.6	0.7	17.7
3	4	2.8	5.5	0.9	8.1	1.2	3.9	0.4	5.7
3	3	4.2	13.1	3.5	10.5	2.4	14.9	2.0	11.8
4	3	4.7	6.5	2.4	3.0	2.3	8.4	1.2	4.0
4	4	2.6	2.5	1.2	3.0	2.2	1.0	1.0	1.2
5	4	11.6	6.7	3.3	4.3	7.1	3.5	2.0	2.4
6	2d	7.1	214.7	3.0	181.8	3.7	100.4	1.6	85.0
6	4	5.9	25.6	1.3	12.3	2.5	12.9	0.6	6.1
6	2u	0.0	394.1	0.0	333.6	0.0	227.5	0.0	192.6
6	1/2	165.2	231.8	464.9	302.4	108.4	210.8	305.1	275.1
7	2	0.0	14.8	0.0	4.9	0.0	8.9	0.0	3.0
7	4/6	0.0	4.2	0.0	2.0	0.0	4.2	0.0	2.0
9	4/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	3/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix F7.--Densities and biomasses of all rainbow trout that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m^2/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	33.5	128.2	41.7	46.8	8.5	25.7	6.4	5.4
1	4/5	36.5	157.0	15.6	66.2	12.9	35.8	4.3	8.7
1	3/5	78.9	106.5	25.6	37.1	25.0	18.9	5.5	4.1
2	3	21.3	209.0	43.6	138.6	4.7	44.1	5.0	15.3
2	4	4.9	177.1	3.8	121.1	1.7	46.2	1.0	18.4
2	2	0.0	97.1	0.0	161.9	0.0	16.7	0.0	10.9
3	4	0.0	23.0	0.0	18.6	0.0	4.6	0.0	2.6
3	3	0.0	31.0	0.0	14.1	0.0	9.8	0.0	2.6
4	3	1.2	27.6	0.3	10.2	1.0	9.6	0.3	2.4
4	4	0.0	12.3	0.0	8.2	0.0	2.8	0.0	1.3
5	4	1.2	11.5	0.2	4.8	1.0	2.7	0.1	0.7
6	2d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	4	0.0	16.3	0.0	6.4	0.0	3.1	0.0	1.1
6	2u	0.0	5.9	0.0	1.1	0.0	1.8	0.0	0.3
6	1/2	4.3	21.9	15.7	140.8	2.0	0.5	2.5	2.9
7	2	0.0	11.1	0.0	4.7	0.0	1.9	0.0	0.4
7	4/6	0.0	25.8	0.0	4.0	0.0	3.8	0.0	0.6
9	4/6	0.0	329.6	0.0	252.5	0.0	61.0	0.0	38.5
10	3/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix F8.--Densities and biomasses of juvenile rainbow trout (2 to 8 inches) that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	24.9	93.6	38.5	41.1	2.8	4.0	4.3	1.8
1	4/5	12.0	105.5	8.8	53.8	1.5	5.8	1.1	2.9
1	3/5	45.2	74.1	20.4	31.9	5.1	3.8	2.3	1.6
2	3	16.5	144.3	40.6	121.1	1.1	7.3	2.8	6.2
2	4	1.1	119.4	2.0	104.1	0.1	8.4	0.2	7.3
2	2	0.0	75.3	0.0	153.3	0.0	3.2	0.0	6.5
3	4	0.0	13.8	0.0	15.3	0.0	1.3	0.0	1.4
3	3	0.0	16.6	0.0	11.4	0.0	1.6	0.0	1.1
4	3	0.0	14.1	0.0	7.5	0.0	1.3	0.0	0.7
4	4	0.0	7.2	0.0	6.7	0.0	0.7	0.0	0.7
5	4	0.0	6.7	0.0	4.1	0.0	0.6	0.0	0.3
6	2d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	4	0.0	9.3	0.0	4.4	0.0	1.0	0.0	0.5
6	2u	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	1/2	2.1	21.9	13.2	140.8	0.1	0.5	0.3	2.9
7	2	0.0	7.4	0.0	4.2	0.0	0.4	0.0	0.2
7	4/6	0.0	18.3	0.0	2.9	0.0	0.9	0.0	0.1
9	4/6	0.0	211.1	0.0	198.2	0.0	21.3	0.0	20.0
10	3/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix F9.--Densities and biomasses of adult rainbow trout (larger than 8 inches) that we observed in test and reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site).

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
1	7/3	8.6	34.5	3.2	5.7	5.8	21.7	2.1	3.6
1	4/5	24.5	47.7	6.8	9.2	11.4	30.0	3.2	5.8
1	3/5	33.7	32.4	5.3	5.3	19.9	15.1	3.1	2.5
2	3	4.9	63.1	3.0	15.6	3.5	36.8	2.2	9.1
2	4	3.8	57.7	1.9	17.0	1.5	37.8	0.8	11.1
2	2	0.0	20.7	0.0	6.7	0.0	13.5	0.0	4.4
3	4	0.0	9.2	0.0	3.3	0.0	3.3	0.0	1.2
3	3	0.0	14.5	0.0	2.7	0.0	8.2	0.0	1.6
4	3	1.2	13.6	0.3	2.8	1.0	8.3	0.3	1.7
4	4	0.0	5.1	0.0	1.6	0.0	2.1	0.0	0.6
5	4	1.2	4.8	0.2	0.8	1.0	2.1	0.1	0.3
6	2d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	4	0.0	7.0	0.0	2.0	0.0	2.1	0.0	0.6
6	2u	0.0	5.9	0.0	1.1	0.0	1.8	0.0	0.3
6	1/2	2.1	0.0	2.5	0.0	2.0	0.0	2.3	0.0
7	2	0.0	3.7	0.0	0.5	0.0	1.5	0.0	0.2
7	4/6	0.0	7.5	0.0	1.1	0.0	2.9	0.0	0.4
9	4/6	0.0	96.3	0.0	44.9	0.0	39.4	0.0	18.4
10	3/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix F10.--Densities and biomasses of brook trout that we observed in Silver Bow Creek and its corresponding reference streams, July and August 1991, and September 1992. Weight is in pounds and weighted usable area (WUA) is in m²/site.

Reach	State code	Number/Ha		(Number/WUA)*1000		Weight/Ha		(Weight/WUA)*1000	
		Test	Reference	Test	Reference	Test	Reference	Test	Reference
		All Brook Trout							
7	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	4/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	4/6	0.0	63.0	0.0	66.6	0.0	8.7	0.0	6.8
10	3/6	0.0	558.3	0.0	220.7	0.0	26.8	0.0	11.0
		Juvenile Brook Trout (2-8 in)							
7	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	4/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	4/6	0.0	48.1	0.0	62.0	0.0	4.2	0.0	5.4
10	3/6	0.0	541.7	0.0	211.9	0.0	23.3	0.0	9.1
		Adult Brook Trout (>8 in)							
7	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	4/6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	4/6	0.0	14.8	0.0	4.6	0.0	4.5	0.0	1.4
10	3/6	0.0	16.7	0.0	8.8	0.0	3.6	0.0	1.9

APPENDIX G

Water conductivities in test and reference reaches.

Appendix G.--Mean ± 1 standard deviation, and range (in parentheses) of conductivities (micromhos/cm) from four measurements between 5 April and 3 May 1992 in test and reference reaches.

Test Streams		Reference Streams	
Reach	Statistics	Stream	Statistics
R 1. Turah	322.8 ± 63.9 (247-402)	Rock Creek (lower)	123.8 ± 23.8 (100-155)
R 2. Beavertail	456.5 ± 21.1 (430-475)	Rock Creek (lower)	123.8 ± 23.8 (100-155)
R 2. Bearmouth	455.5 ± 18.6 (434-479)	Rock Ck (upper)	108.5 ± 17.2 (85-124)
R 4. Gold Creek	438.8 ± 32.0 (400-473)	Big Hole River	128.3 ± 13.4 (109-140)
R 6. Deerlodge	546.3 ± 34.3 (497-574)	Flint Creek	273.0 ± 7.9 (265-282)
R 6. Deerlodge	546.3 ± 34.3 (497-574)	Ruby River	638.3 ± 30.9 (603-675)
R 10. Ramsay	449.0 ± 66.5 (402-496)	Bison Creek	128.5 ± 3.5 (126-131)